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L7: Entry 2 of 2 File: DWPI Sep 25, 2002

DERWENT-ACC-NO: 2002-043130
DERWENT-WEEK: 200278

TITLE: Stabilized preparation containing therapeutically active protein, e.g. blood coagulation factor, comprises stabilizing mixture of saccharide and aminoacid to prevent loss of activity during pasteurization

INVENTOR: ROEMISCH, J; STAUSS, H ; STOEHR, H

PATENT-ASSIGNEE:

ASSIGNEE	CODE
AVENTIS BEHRING GMBH	AVET
CENTEON PHARMA GMBH	CENTN

PRIORITY-DATA: 2000DE-1022092 (May 8, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 2002275090 A	September 25, 2002		004	A61K038/46
EP 1153608 A1	November 14, 2001	G	009	A61K038/36
AU 200143739 A	November 15, 2001		000	C07K001/34
CA 2346616 A1	November 8, 2001	E	000	C07K014/745
DE 10022092 A1	November 15, 2001		000	A61K038/36
US 20010051154 A1	December 13, 2001		000	A61K039/395
KR 2001100988 A	November 14, 2001		000	A61K038/00

DESIGNATED-STATES: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
JP2002275090A	May 7, 2001	2001JP-0136122	
EP 1153608A1	April 18, 2001	2001EP-0109549	
AU 200143739A	May 7, 2001	2001AU-0043739	
CA 2346616A1	May 7, 2001	2001CA-2346616	
DE 10022092A1	May 8, 2000	2000DE-1022092	
US20010051154A1	May 7, 2001	2001US-0849343	
KR2001100988A	May 7, 2001	2001KR-0024563	

INT-CL (IPC): A61 K 38/00; A61 K 38/36; A61 K 38/37; A61 K 38/38; A61 K 38/39; A61 K 38/46; A61 K 38/54; A61 K 39/395; A61 K 47/04; A61 K 47/18; A61 K 47/26; C07 K 1/14; C07 K 1/34; C07 K 1/36; C07 K 14/745; C07 K 14/755; C07 K 14/76; C07 K 14/78; C12 N 9/96

ABSTRACTED-PUB-NO: EP 1153608A
BASIC-ABSTRACT:

NOVELTY - A novel protein preparation (A) (free of antithrombin III) is stabilized against loss of activity during pasteurization by addition of a stabilizing mixture of (a) saccharide(s) and (b) one or more aminoacid(s) selected from arginine, lysine, histidine, phenylalanine, tryptophan, tyrosine, aspartic acid (or its salt) and glutamic acid (or its salt).

DETAILED DESCRIPTION - A novel protein preparation (A) (free of antithrombin III) is stabilized against loss of activity during pasteurization by addition of a stabilizing mixture of:

(a) saccharide(s); and

(b) more than 0.5 mol/l of one or more aminoacid(s) selected from arginine, lysine, histidine, phenylalanine, tryptophan, tyrosine, aspartic acid (or its salt) and glutamic acid (or its salt), the aminoacid component optionally further containing glycine and/or glutamine.

An INDEPENDENT CLAIM is included for a method for inactivating viruses in or removing viruses from in a protein preparation, involving subjecting a stabilized protein preparation (A) to:

(i) heat treatment at 40-95 deg. C for 5-50 hours;

(ii) virus removal by filtration or centrifugation; or

(iii) treatment with detergents, bactericides or virucides.

USE - For stabilizing therapeutically active protein preparations (e.g. containing blood coagulation factors) against loss of activity and denaturation during pasteurization, specifically during inactivation or removal of viruses and other pathogens by heat, mechanical or chemical treatment.

ADVANTAGE - The proteins are effectively stabilized, e.g. remaining almost completely undamaged after pasteurization at 40-95 deg. C for 5-50 hours. The specific aminoacid component (b) provides a better stabilizing effect than the other aminoacids (e.g. glycine) used in DE2916711-A.
ABSTRACTED-PUB-NO:

US20010051154A
EQUIVALENT-ABSTRACTS:

NOVELTY - A novel protein preparation (A) (free of antithrombin III) is stabilized against loss of activity during pasteurization by addition of a stabilizing mixture of (a) saccharide(s) and (b) one or more aminoacid(s) selected from arginine, lysine, histidine, phenylalanine, tryptophan, tyrosine, aspartic acid (or its salt) and glutamic acid (or its salt).

DETAILED DESCRIPTION - A novel protein preparation (A) (free of antithrombin III) is stabilized against loss of activity during pasteurization by addition of a stabilizing mixture of:

(a) saccharide(s); and

(b) more than 0.5 mol/l of one or more aminoacid(s) selected from arginine, lysine, histidine, phenylalanine, tryptophan, tyrosine, aspartic acid (or its salt) and glutamic acid (or its salt), the aminoacid component optionally further containing glycine and/or glutamine.

An INDEPENDENT CLAIM is included for a method for inactivating viruses in or removing viruses from in a protein preparation, involving subjecting a stabilized protein preparation (A) to:

(i) heat treatment at 40-95 deg. C for 5-50 hours;

(ii) virus removal by filtration or centrifugation; or

(iii) treatment with detergents, bactericides or virucides.

USE - For stabilizing therapeutically active protein preparations (e.g. containing blood coagulation factors) against loss of activity and denaturation during pasteurization, specifically during inactivation or removal of viruses and other pathogens by heat, mechanical or chemical treatment.

ADVANTAGE - The proteins are effectively stabilized, e.g. remaining almost completely undamaged after pasteurization at 40-95 deg. C for 5-50 hours. The specific aminoacid component (b) provides a better stabilizing effect than the other aminoacids (e.g. glycine) used in DE2916711-A.

CHOSEN-DRAWING: Dwg.0/0

TITLE-TERMS: STABILISED PREPARATION CONTAIN THERAPEUTIC ACTIVE PROTEIN BLOOD COAGULATE FACTOR COMPRISE STABILISED MIXTURE SACCHARIDE AMINOACID PREVENT LOSS ACTIVE PASTEURISATION

DERWENT-CLASS: B04

CPI-CODES: B04-H19; B04-H20A; B04-L05C; B04-N02;

CHEMICAL-CODES:

Chemical Indexing M1 *01*
Fragmentation Code
M423 M431 M782 M905
Specfic Compounds
A063WK A063WM

Chemical Indexing M1 *02*
Fragmentation Code
M423 M431 M782 M905
Specfic Compounds
A10DEK A10DEM

Chemical Indexing M1 *03*
Fragmentation Code
M423 M431 M782 M905
Specfic Compounds
A03QTK A03QTM

Chemical Indexing M1 *04*
Fragmentation Code
M423 M431 M782 M905
Specfic Compounds
A2BGSK A2BGSM

Chemical Indexing M1 *05*
Fragmentation Code
M423 M431 M782 M905
Specfic Compounds
A03QRK A03QRM

Chemical Indexing M1 *06*
Fragmentation Code
M423 M431 M782 M905
Specfic Compounds
A03QQK A03QQM

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L8: Entry 2 of 2 File: DWPI Feb 7, 2001

DERWENT-ACC-NO: 2001-184355
DERWENT-WEEK: 200212

TITLE: Purifying factor VII-activating protease or its precursor, useful for promoting coagulation, comprises performing fractional precipitation or affinity chromatography

INVENTOR: FEUSSNER, A; ROEMISCH, J ; STOEHR, H

PATENT-ASSIGNEE:

ASSIGNEE	CODE
AVENTIS BEHRING GMBH	AVET
CENTEON PHARMA GMBH	CENTN

PRIORITY-DATA: 1999DE-1037218 (August 6, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
EP 1074615 A1	February 7, 2001	G	010	C12N009/64
DE 19937218 A1	February 8, 2001		000	C07K014/435
CA 2315309 A1	February 6, 2001	E	000	C12N009/50
JP 2001086984 A	April 3, 2001		006	C12N009/50
KR 2001049991 A	June 15, 2001		000	C12N009/50

DESIGNATED-STATES: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
EP 1074615A1	July 5, 2000	2000EP-0114348	
DE 19937218A1	August 6, 1999	1999DE-1037218	
CA 2315309A1	August 3, 2000	2000CA-2315309	
JP2001086984A	August 4, 2000	2000JP-0236805	
KR2001049991A	August 4, 2000	2000KR-0045204	

INT-CL (IPC): A61 K 38/43; A61 K 38/46; A61 K 47/02; A61 K 47/10; A61 K 47/18; A61 K 47/20; A61 K 47/22; A61 K 47/26; A61 K 47/42; A61 L 33/00; A61 P 7/02; A61 P 7/04; A61 P 9/10; A61 P 9/14; A61 P 17/02; B01 D 15/08; C07 K 1/18; C07 K 1/22; C07 K 14/435; C12 N 9/14; C12 N 9/50; C12 N 9/64; C12 N 9/96; C12 Q 1/37; C12 Q 1/56; G01 N 30/48; G01 N 33/531; G01 N 33/573; G01 N 33/86

ABSTRACTED-PUB-NO: EP 1074615A
BASIC-ABSTRACT:

NOVELTY - Purifying factor VII-activating protease (I) and/or its precursor (Ia)

comprising using one or more affinity chromatography separations and/or fractional precipitations, is new.

DETAILED DESCRIPTION - Purifying factor VII-activating protease (I) and/or its precursor (Ia) comprising using one or more:

(a) affinity chromatography separations which involves adsorption on:

(i) calcium phosphate/hydroxylapatite;

(ii) a hydrophobic matrix;

(iii) a chelating matrix;

(iv) a matrix carrying heparin or a related substance; and/or

(v) a matrix carrying antibodies or their Fab/Fab2 fragments specific for (I); and/or

(b) fractional precipitations, is new.

An INDEPENDENT CLAIM is also included for a reagent containing (I) and/or (Ia) and at least one protein stabilizer (II).

ACTIVITY - Procoagulant; antithrombotic; vulnerary; cardiant; cerebroprotective. No supporting data is given.

MECHANISM OF ACTION - Factor VII and prourokinase activator; factor V and VIII inactivator.

USE - (I) and/or (Ia), when formulated with protein stabilizers (II) are used to:

(1) promote coagulation in subjects with a tendency to bleed or have a deficiency in the endogenous coagulation pathway (e.g. factor VIII by-pass activity);

(2) treat or prevent thrombotic complications in subjects with e.g. cardiac infarct, angina pectoris and stroke etc. or in subjects with inherited or acquired (I) deficiency;

(3) to promote wound healing (optionally as part of a fibrin glue or dressing, or in combination with growth factors);

(4) coat the surface of implanted metal or plastic devices, e.g. artificial heart valves, blood vessels and artificial feeding tubes etc.; and

(5) as assay reagents.

(I) is particularly used where a rapid effect is required. (Ia) is more suitable for medium- or long-term prevention or treatment.

ADVANTAGE - (I) and (Ia) can be purified together or separately. Products of a high purity are achieved with good yields.

CHOSEN-DRAWING: Dwg.0/1

TITLE-TERMS: PURIFICATION FACTOR ACTIVATE PROTEASE PRECURSOR USEFUL PROMOTE COAGULATE COMPRISE PERFORMANCE FRACTION PRECIPITATION AFFINITY CHROMATOGRAPHY

DERWENT-CLASS: A96 B04 D16 P34

CPI-CODES: A99-A; B04-L05C; B11-C07A6; B11-C08D2; B14-F01D; B14-F02D1; B14-F08; B14-N17B; D05-H10; D05-H11; D05-H13;

CHEMICAL-CODES:

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L4: Entry 15 of 16 File: JPAB Jan 25, 2000

PUB-NO: JP02000023696A
DOCUMENT-IDENTIFIER: JP 2000023696 A
TITLE: PROTEASE FOR ACTIVATING CLOTTING FACTOR VII

PUBN-DATE: January 25, 2000

INVENTOR-INFORMATION:
NAME COUNTRY
ROEMISCH, JUERGEN DR
STOEHR, HANS-ARNOLD
FEUSSNER, ANNETTE

ASSIGNEE-INFORMATION:
NAME COUNTRY
CENTEON PHARMA GMBH

APPL-NO: JP11116411
APPL-DATE: April 23, 1999

INT-CL (IPC): C12 Q 1/56; A61 K 31/00; A61 K 38/46; C12 N 9/50; C12 Q 1/37; G01 N 33/566; G01 N 33/573

ABSTRACT:

PROBLEM TO BE SOLVED: To provide a protease for activating the clotting factor VII, to provide a method for isolating it, detecting it and inactivating it, and to provide medical preparations comprising the protease.

SOLUTION: This protease and its zymogen have the following properties: (1) being inhibited by the existence of aprotinin; (2) increasing the activity due to calcium ions and/or heparin (or its analogue); (3) showing one or plural bands in the range of 50-75 kDa molecular weight in subsequently dyeing in a nonreduced state and one or plural bands in the range of 10-35 kDa molecular weight in a reduced state respectively according to a SDS-PAGE method; (4) activating the blood coagulation factor VII. The protease and its zymogen are prepared by subjecting blood plasma or prothrombin complex (PPSB) concentrate to anion exchange chromatography preliminarily and then by subjecting the product to affinity chromatography using heparin (or its analogue) or dextran sulfuric acid.

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L9: Entry 1 of 1 File: DWPI Mar 4, 2003

DERWENT-ACC-NO: 1999-602901
DERWENT-WEEK: 200320

TITLE: New protease for activating blood clotting factor VII, useful for treating hemorrhage prophylaxis or hemostasis

INVENTOR: FEUSSNER, A; ROMISCH, J ; STOHR, H ; ROEMISCH, J ; STOEHR, H

PATENT-ASSIGNEE:

ASSIGNEE	CODE
AVENTIS BEHRING GMBH	AVET
CENTEON PHARMA GMBH	CENTN

PRIORITY-DATA: 1999DE-1003693 (February 1, 1999), 1998DE-1018495 (April 24, 1998), 1998DE-1027734 (June 22, 1998), 1998DE-1051332 (November 6, 1998), 1998DE-1051335 (November 6, 1998), 1998DE-1051336 (November 6, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 6528299 B1	March 4, 2003		000	C12N009/50
EP 952215 A2	October 27, 1999	E	024	C12N009/64
DE 19903693 A1	October 28, 1999		000	C12N009/48
AU 9923935 A	November 4, 1999		000	A61K038/48
CA 2269109 A1	October 24, 1999	E	000	C12N009/96
JP 2000023696 A	January 25, 2000		018	C12Q001/56
KR 99083444 A	November 25, 1999		000	C12N009/50
AU 748221 B	May 30, 2002		000	A61K038/48

DESIGNATED-STATES: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
US 6528299B1	April 21, 1999	1999US-0295316	
EP 952215A2	April 8, 1999	1999EP-0106913	
DE 19903693A1	February 1, 1999	1999DE-1003693	
AU 9923935A	April 23, 1999	1999AU-0023935	
CA 2269109A1	April 23, 1999	1999CA-2269109	
JP2000023696A	April 23, 1999	1999JP-0116411	
KR 99083444A	April 23, 1999	1999KR-0014636	
AU 748221B	April 23, 1999	1999AU-0023935	
AU 748221B		AU 9923935	Previous Publ.

INT-CL (IPC): A61 K 31/00; A61 K 38/00; A61 K 38/43; A61 K 38/46; A61 K 38/48; A61 K

38/49; A61 K 38/55; A61 K 38/57; A61 L 15/38; C07 K 14/745; C07 K 16/40; C12 N 9/48; C12 N 9/50; C12 N 9/64; C12 N 9/96; C12 Q 1/37; C12 Q 1/56; G01 N 33/566; G01 N 33/573; G01 N 33/577; G01 N 33/86

ABSTRACTED-PUB-NO: EP 952215A
BASIC-ABSTRACT:

NOVELTY - A protease (I) for activating the blood clotting factor VII is new and:

(a) is inhibited by aprotinin;

(b) its activity is increased by calcium ions and/or heparin (or related substances);

(c) in SDS-PAGE, in non-reduced state has one or more bands in the molecular weight range from 50-75 kDa and in the reduced state has a band at 40-55 kDa and one or more bands in the molecular weight range from 10-35 kDa.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a process for obtaining or removing (I) or proenzyme, comprising obtaining it from blood plasma or prothrombin (PPSB) concentrates after prior anion exchange chromatography by means of affinity chromatography using heparin or a substance related to heparin or dextran sulfate;

(2) a reagent (II) for the immunological detection of (I) or the proenzyme which contains a poly- or monoclonal antibody against (I) or proenzyme;

(3) a reagent for the diagnostic/analytical purposes which contains (I) and/or the proenzyme optional together with activities of the proenzyme;

(4) a reagent for the detection of factor VII which contains (I)/proenzyme optionally together with protease activity enhancing compounds;

(5) a test system for the qualitative and quantitative detection of (I) or its proenzyme comprising:

(a) its activity inactivating the blood clotting factors VIII/VIIIa or V/Va; or

(b) its activity reducing the blood clotting times in global clotting tests; or

(c) its activity activating plasminogen activators;

(d) its activity activating FVII;

(6) an assay system comprising (I) and/or a mixture of the proenzyme and appropriate proenzyme activators is used to test the prothrombin time substituting tissue factor/thromboplastin or the functionality of plasminogen activators and for the quantification of the single chain plasminogen activator forms;

(7) a stabilized factor V and stabilized factor VIII preparation which is free of the inactive factor VIII and factor V fragments formed due to the proteolytic degradation as a result of the inhibition of the protease activating the blood clotting factor VI;

(8) a stabilized solution of (I) or the proenzyme which is adjusted to a pH of 4.0-9.0 by the addition of a buffer and/or contains ethylene glycol or glycerol in an amount of 5-80 wt.%;

(9) process for the preparation of a pharmaceutical preparation comprising (I) and/or the enzyme comprising:

(i) in a pH range of 3.5-8.0;

(ii) with addition of one or more amino acids, more than 0.01 mol/l; and/or

(iii) with addition of a sugar or a combination of a number of sugars, more than 0.05 g/ml; and/or

(iv) with addition of one or more substances; which are able to complex calcium ions under pasteurization conditions; and

(10) the use of (I) or its proenzyme, optionally together with proenzyme activators prepared from blood plasma or prothrombin complex (PPSB) concentrates or expressed recombinantly or transgenically for the promotion of wound healing and hemostasis, as an additive of a fibrin adhesive or fleece or other release system which is suitable for rapid wound closure based on fibrin, for substitution in inborn or acquired deficiency states of this protease or its proenzyme, in the presence of antibodies against the blood clotting factor VIII or for the in vitro activation of factor VII.

ACTIVITY - Coagulant; Anticoagulant; Antithrombotic.

MECHANISM OF ACTION - The protease (I) activates Factor VII.

USE - The protease (I) is useful for:

(1) the preparation of a pharmaceutical for activating the blood clotting factor VII, and/or its proenzyme, adequate for the dissolution of fibrin containing thrombi;

(2) the preparation of a pharmaceutical and/or its protease and optionally further comprises single chain or two chain plasminogen activators (PA) and/or anticoagulants; and

(3) the preparation of a pharmaceutical for decreasing the coagulability of the blood which contains a protease inhibitor e.g. aprotinin and/or a C1 inhibitor and/or alpha 2-Antiplasmin and/or Inter-Trypsin-Inhibitor and/or AT III/heparin for the inhibition of the protease and/or its proenzyme (all claimed).

The pharmaceutical compositions comprising (I) is useful as a blood coagulating agent for general hemorrhage prophylaxis or for staunching hemorrhages e.g. treating patients suffering from hemophilia A. (I) is also useful as a diagnostic, especially for detecting the presence and activation of factor VII qualitatively and quantitatively using (I). (I) is also useful for developing an agent which diminishes the ability of the blood to coagulate. (I) in conjunction with plasminogen activators the pharmaceutical preparations are useful for treating thromboembolic diseases or complications e.g. leg vein thrombosis, cardiac infarction or strokes.

ADVANTAGE - The protease (I) exhibits particularly high amidolytic activity towards the peptide substrate S2288 (HD-isoleucyl-L-prolyl-L-arg- inine-pNA). The amidolytic activity is efficiently inhibited by aprotinin.

CHOSEN-DRAWING: Dwg.0/3

TITLE-TERMS: NEW PROTEASE ACTIVATE BLOOD CLOT FACTOR USEFUL TREAT HAEMORRHAGE PROPHYLACTIC HAEMOSTATIC

DERWENT-CLASS: B04 D16 P34 S03

CPI-CODES: B04-C02E; B04-D01; B04-G03; B04-G06; B04-G21; B04-G22; B04-L05C; B10-B02; B11-C08E3; B12-K04A; B14-F01B; B14-F02D1; B14-F08; B14-N17B; D05-A02C; D05-C03C; D05-H09;

EPI-CODES: S03-E14H4; S03-E14H5;

CHEMICAL-CODES:

L11: Entry 216 of 236 File: USPT Feb 18, 1997

DOCUMENT-IDENTIFIER: US 5604202 A
TITLE: Use of NGF growth factors to treat drug-induced neuropathy

Drawing Description Text (17):
Therapeutic formulations of neuronotrophic factors are prepared for storage by mixing the factor having the desired degree of purity with physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences, 16th ed., 1980, Mack Publishing Co., edited by Oslo, et al., the disclosure of which is incorporated herein by reference) in the form of a lyophilized cake or aqueous solutions. Acceptable carriers, excipients and stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phospate, citrate, succinate, acetate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as ethylenediaminetetraacetic acid (EDTA); sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronics, or polyethylene glycol.

L11: Entry 218 of 236 File: USPT Dec 31, 1996

DOCUMENT-IDENTIFIER: US 5589363 A
TITLE: DNA encoding tissue factor mutants useful for the treatment of myocardial infarction and coagulopathic disorders

Detailed Description Text (149):
Therapeutic formulations of tissue factor protein mutant are prepared for storage by mixing tissue factor protein mutant having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, supra), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosacchaddes, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, octylglucoside, Pluronics or polyethylene glycol (PEG). Tissue factor protein mutant may also be admixed with phospholipids, including PS and PC, or used in conjunction with liposomes.

L11: Entry 219 of 236 File: USPT Dec 10, 1996

DOCUMENT-IDENTIFIER: US 5583107 A
TITLE: Agents affecting thrombosis and hemostasis

Detailed Description Text (71):
Therapeutic formulations of the blood factors of this invention, or of a blood factor antibody are prepared for storage by mixing the blood factor polypeptide or antibody having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, supra), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counter ions such as sodium; and/or nonionic surfactants such as Tween, Pluronics or polyethylene glycol (PEG).

L11: Entry 217 of 236 File: USPT Jan 14, 1997

DOCUMENT-IDENTIFIER: US 5593674 A
TITLE: Plasma carboxypeptidase

Detailed Description Text (116):
Therapeutic formulations of PCPB for treating blood clotting disorders are prepared for storage by mixing PCPB having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, supra), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronics or polyethylene glycol (PEG).

L4: Entry 14 of 16 File: JPAB Feb 6, 2001

PUB-NO: JP02001029098A
DOCUMENT-IDENTIFIER: JP 2001029098 A
TITLE: ACTIVITY MEASUREMENT OF FACTOR VII-ACTIVATING PROTEASE CONTAINED IN PROTEIN SOLUTION

PUBN-DATE: February 6, 2001

INVENTOR-INFORMATION:
NAME COUNTRY
ROEMISCH, JUERGEN DR
FEUSSNER, ANNETTE
STOEHR, HANS-ARNOLD

ASSIGNEE-INFORMATION:
NAME COUNTRY
AVENTIS BEHRING GMBH

APPL-NO: JP2000174893
APPL-DATE: June 12, 2000

INT-CL (IPC): C12 Q 1/37; G01 N 33/573; G01 N 33/86

ABSTRACT:

PROBLEM TO BE SOLVED: To provide a method for measuring protease activity activating blood coagulation factor VII in a protein solution.

SOLUTION: This method is to incubate a protein solution containing protease and/or its pro-enzyme together with a solid phase previously combined with antibody to the protease, to wash the solid phase and then to incubate the protease and/or its pro-enzyme immobilized on the solid phase together with a reagent enabling their activities to be measured. The method enables blood coagulation factor VII-activating protease to conveniently and specifically be measured and is useful for early detection of disease.

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L4: Entry 13 of 16 File: JPAB Apr 3, 2001

PUB-NO: JP02001086984A
DOCUMENT-IDENTIFIER: JP 2001086984 A
TITLE: PREPARATION OF PROTEASE WHICH ACTIVATES BLOOD COAGULATION FACTOR VII, PROENZYME THEREOF, OR MIXTURE OF THEM IN PURE FORM BY AFFINITY CHROMATOGRAPHY

PUBN-DATE: April 3, 2001

INVENTOR-INFORMATION:
NAME COUNTRY
ROEMISCH, JUERGEN DR
FEUSSNER, ANNETTE
STOEHR, HANS-ARNOLD

ASSIGNEE-INFORMATION:
NAME COUNTRY
AVENTIS BEHRING GMBH

APPL-NO: JP2000236805
APPL-DATE: August 4, 2000

INT-CL (IPC): C12 N 9/50; A61 K 38/43; A61 K 38/46; A61 K 47/02; A61 K 47/10; A61 K 47/18; A61 K 47/20; A61 K 47/22; A61 K 47/26; A61 K 47/42; A61 L 33/00; A61 P 7/02; A61 P 7/04; A61 P 9/10; A61 P 9/14; A61 P 17/02; C12 N 9/96; G01 N 30/48; G01 N 33/531

ABSTRACT:

PROBLEM TO BE SOLVED: To provide a method for preparing protease which activates blood coagulation factor VII, proenzyme thereof, or a mixture of them in a pure form by the affinity chromatography.

SOLUTION: This method comprises one affinity chromatographic step or more utilizing adsorption on calcium phosphate/hydroxyapatite, hydrophobic matrix, chelate matrix, matrix obtained by immobilizing a heparin-related substance such as heparin, heparan sulfate, and dextran sulfate, and/or immobilized monoclonal antibody or polyclonal antibody against a protein to be isolated, or matrix coated by its F(ab) or F(ab)2 fragment, and/or fractional precipitation.

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L4: Entry 12 of 16 File: JPAB Apr 10, 2001

PUB-NO: JP02001095563A
DOCUMENT-IDENTIFIER: JP 2001095563 A
TITLE: PREPARATION OF PROTEASE ACTIVATING BLOOD COAGULATION FACTOR VII, ITS PROENZYME OR MIXTURE OF BOTH PROTEINS IN PURIFIED STATE BY ION EXCHANGE CHROMATOGRAPHY

PUBN-DATE: April 10, 2001

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APPL-DATE: August 4, 2000

INT-CL (IPC): C12 N 9/50; A61 K 38/43; A61 K 38/46; A61 K 47/02; A61 K 47/10; A61 K 47/12; A61 K 47/18; A61 K 47/20; A61 K 47/22; A61 K 47/26; A61 K 47/34; A61 K 47/42; A61 L 27/00; A61 L 33/00; A61 P 7/04; A61 P 9/10; C12 N 9/96; G01 N 30/02; G01 N 30/48; G01 N 33/531; C12 Q 1/37; C12 Q 1/56; G01 N 33/573

ABSTRACT:

PROBLEM TO BE SOLVED: To provide a process for producing protease activating blood coagulation factor VII, its proenzyme or a mixture of both proteins in purified state by ion-exchange chromatography.

SOLUTION: The process for the preparation of the proteins in purified state comprises (a) the anion- and/or cation-exchange chromatography at a pH lower than the isoelectric point of the protein to be separated or (b) a combination of the anion- or cation-exchange chromatography with affinity chromatography and/or fractional precipitation at pH 2.5-9.0, preferably pH 2.5-7.2 (the affinity chromatography is carried out by using calcium phosphate/ hydroxyapatite, a hydrophobic matrix, a chelate matrix or a matrix coated with immobilized monoclonal antibody or polyclonal antibody to the protein to be separated or its F(ab) or F(ab)2 fragment).

L7: Entry 32 of 78 File: USPT Sep 20, 1994

DOCUMENT-IDENTIFIER: US 5348738 A
TITLE: Oral composition with active water insoluble polymer

Detailed Description Text (33):
In the oral composition of the present invention, as a pharmacologically active component, there can be formulated bactericides (e.g., cetylpyridinium chloride, chlorohexidine salts, triclosan, etc.), enzymes (e.g., dextranase, amylase, protease, mutanase, lysozyme, lytic enzyme, etc.), alkaline metal monofluorophosphate (e.g., sodium monofluorophosphate, potassium monofluorophosphate, etc.), fluorides (e.g., sodium fluoride, stannous fluoride, etc.), tranexamic acid, epsilon-aminocapronic acid, aluminium chlorohydroxylallantoin, dihydrocholesterol, glycyrrhizine salts, glycyrrhetic acid, glycerophosphate, chlorophyll, sodium chloride, caropeptide, water-soluble inorganic phosphoric acid compounds alone or in combination thereof. The amounts of these pharmacologically active components to be formulated are the same as those in conventional oral compositions. Any person skilled in the art can appropriately determine the amounts depending upon a particular use of the composition.

L17: Entry 20 of 46 File: USPT Jul 13, 1982

DOCUMENT-IDENTIFIER: US 4339432 A
TITLE: Oral mouthwash containing zinc and glycine

Brief Summary Text (12):
The zinc salts, according to the present invention, will generally be present in the oral composition in an amount of from about 0.04% to about 2.0%. In the case of mouthwash products, the zinc salt may be added at a level of about 0.04% to about 0.7% by weight of soluble zinc ion, with 0.04% being the approximate minimum active concentration and 0.7% being the approximate concentration at which astringency becomes objectionable. The preferred concentration of zinc ion in a mouthwash is 0.1%-0.3%, and 0.2% to 3% in a toothpaste. By adding glycine and raising the pH, however, the level of zinc may be raised without objectionable astringency.

Detailed Description Paragraph Table (6):

Toothpaste Ingredient % weight
Silica Xerogel (Syloid 63) 10.00 Humectant (Sorbitol) 40.00 Sodium Lauryl Sulfate (21% in glycerine) 7.00 Bodying Agent (Na Carboxymethyl- cellulose) 1.00 Flavor, color 1.50 Zinc chloride 1.00 Glycine 2.00 NaHCO.sub.3 to pH 6.0 Water Balance to 100%

Detailed Description Paragraph Table (7):

Toothpaste Ingredient % weight
Silica Xerogel (Syloid 63) 15.00 Powdered Polyethylene* 5.00 Na Carboxymethylcellulose .80 Glycerol 65.00 Saccharin .20 Zinc chloride .60 Glycine 1.50 Flavor 1.30 Coloring agent .25 Foaming agent .65 NaOH to pH 6.3 Water Balance to 100%

*High density polyethylene powder, average particle size 8-9 microns.

L17: Entry 21 of 46 File: USPT Oct 20, 1981

DOCUMENT-IDENTIFIER: US 4296095 A
TITLE: Dental and mouth care preparations

Brief Summary Text (33):
Such toothpaste should contain 0.1 to 10 weight % and preferably 1 weight % based on the total weight of the sum of all ingredients of the paste of lysine ester or salt in order to obtain the effect of preventing dental plaque and tartar formation on the teeth in using the common quantity of toothpaste (the formation of at least a monomolecular layer of the lysine compound on the teeth is necessary to bring this effect).

Detailed Description Paragraph Table (1):
TABLE II

	Wt. %
Example 1-- Toothpaste Lysine palmityl ester dihydrochloride	1.0
Aluminum oxyhydrate	55.0
Glycerol	20.0
Tragacanth	1.0
Polyoxyethylene sorbitane mono-oleate ("Tween 80")	1.0
Sodium saccharin	0.2
Aroma (peppermint oil)	1.0
Water	20.8
Example 2-- Mouth wash Lysine palmityl ester dihydrochloride	1.0
Sorbitol, 70%	5.0
Reaction product of 1 mole hydr. castor oil with about 40 moles ethylene oxide ("Cremophor RH 40", BASF)	2.0
Aromatics and flavors	1.0
Ethanol, 95%	30.0
Water	61.0
Example 3-- Chewing gum Chicle gum	20.6
Cane sugar	40.0
Dextrose	22.0
Corn syrup, 43.degree. Be	15.7
Glycerol	0.8
Peppermint oil	0.7
Lysine stearyl ester dihydrofluoride	0.2

L15: Entry 98 of 134 File: USPT Apr 4, 1978

DOCUMENT-IDENTIFIER: US 4082841 A
TITLE: Dentifrice

Brief Summary Text (38):
Humectants are desirable in a toothpaste. These will usually be such compounds as glucose, honey, glycerol, propylene glycol, sorbitol, polyethylene glycol 400, and other polyhydric alcohols, and may be present in the composition in amounts up to about 80% by weight.

L15: Entry 99 of 134 File: USPT Feb 28, 1978

DOCUMENT-IDENTIFIER: US 4076549 A
TITLE: Amorphous precipitated siliceous pigments for cosmetic or dentifrice use and methods for their preparation

Brief Summary Text (32):
If the pigments of the invention are used in toothpaste compositions, the dentifrice (if in the form of a paste) may contain humectant materials and binders to give the dentifrice a smooth texture and good flowability. Glycerine, sorbitol, corn syrup, glucose and the like may be used as carriers. Examples of binders include gum tragacanth, sodium carboxymethylcellulose and the like. The above materials as well as specific formulations and ingredients of toothpaste compositions are well known in the art and are disclosed in numerous publications and e.g., in U.S. Pat. Nos. 2,994,642 and 3,538,230.

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L13: Entry 28 of 33 File: USPT Oct 20, 1981

DOCUMENT-IDENTIFIER: US 4296095 A
TITLE: Dental and mouth care preparations

Detailed Description Paragraph Table (1):
TABLE II

	Wt. %
Example 1-- Toothpaste	
Lysine palmityl ester dihydrochloride	1.0
Aluminum oxyhydrate	55.0
Glycerol	20.0
Tragacanth	1.0
Polyoxyethylene sorbitane mono-oleate ("Tween 80")	1.0
Sodium saccharin	0.2
Aroma (peppermint oil)	1.0
Water	20.8
Example 2-- Mouth wash	
Lysine palmityl ester dihydrochloride	1.0
Sorbitol, 70%	5.0
Reaction product of 1 mole hydr. castor oil with about 40 moles ethylene oxide ("Cremophor RH 40", BASF)	2.0
Aromatics and flavors	1.0
Ethanol, 95%	30.0
Water	61.0
Example 3-- Chewing gum	
Chicle gum	20.6
Cane sugar	40.0
Dextrose	22.0
Corn syrup, 43.degree. Be	15.7
Glycerol	0.8
Peppermint oil	0.7
Lysine stearyl ester dihydrofluoride	0.2

L7: Entry 10 of 12 File: USPT May 5, 1998

DOCUMENT-IDENTIFIER: US 5747030 A
TITLE: Pharmaceutical preparation containing plasminogen activators

Abstract Text (1):
The invention relates to pharmaceutical preparations containing plasminogen activators, sugars and tranexamic acid, in the form of a lyophilisate or an injection or infusion solution. In particular, the preparations contain a sugar, phosphate buffer, tranexamic acid as well as a surfactant and the liquid solutions preferably have a pH value of 5.5-6.5.

Brief Summary Text (4):
In the meaning of the invention, in principle, such derivatives of t-PA, particularly those prepared by recombination, are possible as the plasminogen activators, which essentially comprise those protein regions of natural protein that are responsible for the fibrinolysis of the thrombi. Here, such t-PA derivatives may also be used which have deletions or substitutions of single or multiple amino acids in the t-PA sequence.

Brief Summary Text (5):
According to the invention, the following plasminogen activators may be employed, for example: t-PA (e.g., Alteplase), LY 210825 (K2P available from "Syrian hamster" cell lines, Circ. 1990, 82, 930-940); .DELTA.FE3x and .DELTA.FE1x (K1K2P, Blood 1988, 71, 216-219); .DELTA.FEK1 (K2P from C127 mouse cells, J. Cardiovasc. Pharmacol. 1990, 16, 197-209); E-6010 (Jap. Circ. J. 1989, 53, p. 918); t-PA variants (Thromb. Haemost. 1989, 62, p. 542); K2P and D-K2P (Thromb. Haemost. 1989, 62, p. 393); MB-1018 (FK2K2P, Thromb. Haemost. 1989, 62, p. 543); FK2P (FASEB J. 1989, 3, A1031, abstract 4791); .DELTA.1x (Circulation 1988, 4, II-15); K1K2P (Thromb. Res. 1988, 50, 33-41); FK1K2P (J. Biol. Chem. 1988, 263, 1599-1602); TNK variant of t-PA (WO 93/24635); bat-PA (Witt et al., Blood 1992, 79, 1213-1217, and Mullot et al., Arterioscler. Thrombos. 1992, 12, 212-221). In particular, those plasminogen activators are possible which contain the cringle 2 domain ("K2") of the t-PA and/or the serine protease domain ("P"). In this respect, one may exemplify the K2P type t-PA mutein "r-PA" described in EP 0 382 174 (WO 90/09437).

Brief Summary Text (7):
The substantial influence of the sugar proportion on solubility and aggregation of proteins is known from prior art (J. Biol. Chem. 263 (1988), 8832-8837). Thus, it has been determined in EP-B-458 950 that, e.g., a non-glycosylated recombinant plasminogen activator of domain composition K2P has a substantially poorer solubility than glycosylated t-PA derivatives, for example. As a rule, non-glycosylated plasminogen activators such as r-PA dissolve only to a slight extent in buffers conventionally used for the solubilisation of proteins, such as 50 mmoles/l of Na citrate pH 6, 50 mmoles/l of phosphate buffer or physiological NaCl solution. However, utilisation as a therapeutic agent requires plasminogen activators to be present at sufficiently high concentrations, preferably, at a concentration of up to 10 mg/ml.

Brief Summary Text (8):
Increasing the solubility of t-PA from prokaryotes by neutral or slightly alkaline arginine formulations is known from EP-A-0 217 379. However, this procedure suffers from the drawback that good solubility of t-PA from prokaryotes can only be achieved with very high arginine concentrations.

Brief Summary Text (10):
Thus, WO 90/01333 (Invitron) describes the combination of lysine, histidine, arginine with citrate for t-PA and derivatives from bacteria. Citrate is used at 5 mmoles/l, lysine, histidine, arginine at 150 mmoles/l at pH 6. Furthermore, albumin is added.

Brief Summary Text (11):
WO 89/050347 (Invitron) describes the combination of arginine (20-200 mmoles/l) and citrate (20-80 mmoles/l) at pH 5-8.

Brief Summary Text (12):
WO 90/08557 (Genetics Institute) discloses a combination of creatinine with various additives such as histidine, arginine, proline, betaine, choline, imidazole, tryptophan, citrate, optionally with addition of glutamic acid, aspartic acid and succinic acid.

Brief Summary Text (13):
EP 0 297 294 (Behring) discloses the combination of at least two amino acids such as lysine, ornithine, arginine, tranexamic acid and other additives at pH 5-10.

Brief Summary Text (14):
EP 0 156 169 (Asahi) describes ornithine and/or lysine, optionally with addition of citrate, glycine or phosphate, and EP 0 228 862 (Genentech) discloses a formulation containing arginine with or without chloride and with or without detergent.

Brief Summary Text (15):
Likewise, alternative formulations of r-PA in the presence of lysine and lysine analogues, respectively, in solutions buffered with citric acid are described in EP 0 458 950 (Boehringer Mannheim). However, more recent examinations demonstrate that the solubility of r-PA in these formulations as well, is not as yet completely sufficient. It has been determined that in fact, the solubility may be improved by increasing the citrate concentration; however, such formulations are not or only in part tolerated by the veins. Moreover, the high salt concentrations and the low glass temperature (Tg') associated therewith result in the necessity to carry out the lyophilisation of these formulations at temperatures below -45.degree. C. and -50.degree. C. Technically, these temperatures are realizable often only at a very high expenditure, and they require complex control and monitoring elements in order to control and measure the optimum conditions for lyophilisation. Moreover, this involves a relatively high expenditure of energy. Furthermore, for industrial production there are often used rather old lyophilisation plants, which lack the requisite complex measuring and control technology, and where, consequently, as a rule, there can only be guaranteed a working temperature of about -45.degree. C.

Brief Summary Text (17):
The object of the invention is attained by a pharmaceutical preparation containing a plasminogen activator, with the preparation containing at least one sugar and tranexamic acid as pharmaceutical additives. In lyophilised form, the preparation is stable on storage over a prolonged period of time. Likewise, the aqueous injection solution prepared by reconstitution meets this criterion of improved storage stability. In particular, this is the case when the pH value of the solution is adjusted to a value of between 5.5-6.5. As further additives, the pharmaceutical preparations may contain conventional buffer substances or surface-active materials (anionic, cationic or neutral surfactants).

Brief Summary Text (18):
As an example of the plasminogen activators (PA) possible within the meaning of the invention, the plasminogen activator K2P (BM 06.022) described in more detail in the European patent application EP-A-0 382 174 was used. It consists of the cringle 2 (K2) and the protease domain (P) of human t-PA and, due to its expression in Escherichia coli cells, is present in non-glycosylated form.

Brief Summary Text (19):
As sugar, the formulations of the invention preferably contain mono- or disaccharides. As the disaccharides, particularly saccharose, trehalose, maltose or

lactose are possible. In particular, the monosaccharides are galactose or corresponding amino sugars such as, e.g., galactosamine. Preferably, the non-reducing sugars saccharose and trehalose are employed. The sugar concentration in the aqueous injection solution is preferably 40-100 mg/ml, with between 50-70 mg/ml being preferred.

Brief Summary Text (20):
As buffer substances, the pharmaceutical preparations may contain conventional substances possible for such purposes and representing salts of strong acids with weak bases or of weak acids with strong bases. As examples, there may be mentioned: alkali salts of phosphoric acid, tartaric acid, malic acid and the like. Amino acids are also possible. Preferably, the preparations according to the invention contain phosphate buffer. In particular, the concentration of phosphate buffer in the aqueous solution ready for injection is 50-300 mmoles/l, preferably 80-220 mmoles/l and, particularly preferred, 130-170 mmoles/l. As a phosphate buffer, disodium or dipotassium hydrogen phosphate is preferably used, which is adjusted to the respective pH value using phosphoric acid.

Brief Summary Text (21):
As solubiliser, the preparations according to the invention contain tranexamic acid (TES). The concentration of tranexamic acid in the aqueous solution ready for injection is preferred to be 1-50 mmoles/l, preferably 8-12 mmoles/l. Particularly advantageous is the use of a concentration of 10 mmoles/l. Surprisingly, tranexamic acid has been found to increase the solubility of plasminogen activators, particularly of non-glycosylated plasminogen activators in an aqueous medium. For instance, in the case of the plasminogen activator derivative r-PA, solubility was increased at least by a factor of 1.5 with respect to a solution free of tranexamic acid. The factor for the solubility increase preferably ranges from 2-3. This effect, above all, is advantageous within the scope of the preparation of lyophilised pharmaceutical preparations of plasminogen activators since, due to the possibility of increasing the concentration of the plasminogen activator in the solutions used, the amount of water to be used in the production process may be reduced significantly and, on the whole, a production is achieved which is energy-saving and more cost effective. In particular, the lyophilisation periods in the production process are reduced.

Brief Summary Text (22):
As surfactants, non-ionic, anionic or cationic surfactants, preferably, however, non-ionic surfactants such as Tween 80 or Tween 20 may be used. The surfactant concentration is 0.005-0.1% (w/v) and preferably 0.01%.

Brief Summary Text (27):
In addition, the invention is directed to the use of a specified mixture of pharmaceutical adjuvants consisting of the group sugar and tranexamic acid for the longterm stabilisation of plasminogen activators. In particular, in order to achieve good storage stability, a combination of the adjuvants sugar, phosphate buffer, tranexamic acid, and surfactant is especially advantageous.

Brief Summary Text (30):
In the meaning of the present invention, relatively high glass temperatures can be attained for the frozen solutions especially when a potassium salt such as dipotassium hydrogen phosphate or potassium dihydrogen phosphate is used as the phosphate buffer for the solutions required for lyophilisation. In this regard, in particular, dipotassium hydrogen phosphate at a concentration of from 20-40 mg/ml, preferably 20-30 mg/ml can be used. The increase in glass temperature can be enhanced by adding saccharose. In this connection, saccharose is added preferably at a concentration of from 60-90 mg/ml, in particular, 60-80 mg/ml. The use of a solution containing about 26 mg/ml of dipotassium hydrogen phosphate (K.sub.2 HPO.sub.4 .times.12 H.sub.2 O) and about 70 mg/ml of saccharose is particularly preferred. The pH value of the solution is adjusted to a value of 6 using, preferably, 85%-phosphoric acid (about 12 mg/ml). The solution further contains about 0.5-5 mg/ml, preferably 1-2 mg/ml tranexamic acid, in particular, about 1.6 mg/ml. Besides this, the solution may contain, in addition, surfactants such as Tween 80, which is applied preferably at a concentration of from 0.01-0.3 mg/ml, in particular, about 0.1 mg/ml.

Brief Summary Text (32):
Another advantage of the preparation according to the invention is that when producing the lyophilisates, due to the increase in solubility of non-glycosylated plasminogen activators, in particular, in the case of the type K2P, K1K2P or P type muteins, achieved by the addition of tranexamic acid, one may start with smaller volumes (for example, of about 5 ml per single administration form) of solutions to be frozen, so that the lyophilisation period is significantly reduced with respect to solutions hitherto used for the preparation of lyophilisates (for example, of about 10 ml per administration form). Preferably, one starts with solutions containing the active substance at a concentration about twice as high as compared to the aqueous administration form ready for injection so that aqueous solutions of 5 ml can be used instead of the hitherto conventional solutions of 10 ml. "It is most preferred where, prior to lyophilization, the aqueous solution contains the active substance at a concentration which is up to three times higher than the concentration of a ready-to-use aqueous injection or infusion solution."

Detailed Description Text (3):
r-PA (BM 06.022) was adjusted to a protein concentration (C.sub.prot.) of 6 mg/ml (ultrafiltration over an Amicon YM 10 membrane) and dialysed against the buffers as indicated. Subsequently, the samples were adjusted to C.sub.prot. =4 mg/ml and were a) left unchanged, b) filled up with 0.01% Tween 80, and c) with 0.01% Tween 20.

Detailed Description Text (7):
The present data shows that without addition of detergents, there is substantial increase in light scattering of the sample under mechanical stress (FIG. 1). By adding detergents, the increase can be suppressed to the largest extent. Within the scope of measuring accuracy, no difference between Tween 80 and Tween 20 is notable.

Detailed Description Text (11):
r-PA was dialysed against the buffer (without Tween 80) indicated in Table 1, adjusted to C.sub.prot. =4 mg/ml, filled up with Tween 80 to C=0.01%, portioned, and sterile filtrated. Each time 9 samples were frozen at -20.degree. and -70.degree. C., respectively. On the days as indicated in the Table all of the samples were thawed (15 min at 25.degree. C. in a water bath) except for the control. Each time one sample was analysed (C.sub.prot. and amidolytic activity). The rest of the samples were frozen at -20.degree. C. and -70.degree. C., respectively. After completion of the series the activity of the non-stressed sample was determined.

Detailed Description Text (17):
r-PA (BM 06.022) was concentrated over an Amicon YM 10 membrane to 5 mg/ml and dialysed against the buffer (without Tween 80) indicated in Table 2. The dialysates were adjusted to C.sub.prot. =4 mg/ml, filled up with Tween 80 to C=0.01%, portioned, and stored at -20.degree. C. and 4.degree. C. After 7, 14, 20 and 30 days, amidolytic activity and protein concentration of the stressed samples were determined.

Detailed Description Paragraph Table (1):
A) 150 mM of Na.sub.2 HPO.sub.4 /H.sub.3 PO.sub.4, pH 6.0 10 mM of TES 50 mg/ml of saccharose a) without detergent b) with 0.01% of Tween 80 c) with 0.01% of Tween 20 B) 150 mM of Na.sub.2 HPO.sub.4 /H.sub.3 PO.sub.4, pH 6.0 10 mM of TES 50 mg/ml of trehalose a) without detergent b) with 0.01% of Tween 80 c) with 0.01% of Tween 20 C) 150 mM of K.sub.2 HPO.sub.4 /H.sub.3 PO.sub.4, pH 6.0 10 mM of TES 50 mg/ml of saccharose a) without detergent b) with 0.01% of Tween 80 c) with 0.01% of Tween 20 D) 150 mM of K.sub.2 HPO.sub.4 /H.sub.3 PO.sub.4, pH 6.0 10 mM of TES 50 mg/ml of trehalose a) without detergent b) with 0.01% of Tween 80 c) with 0.01% of Tween 20

Detailed Description Paragraph Table (2):
TABLE 1

150 mM of Na.sub.2 HPO.sub.4 /H.sub.3 PO.sub.4, pH 6.0 10 mM of TES 50 mg/ml of saccharose 0.01% of Tween 80 -20.degree. C. -70.degree. C. C.sub.prot. AA C.sub.prot. AA Day [mg/ml] [MU/ml] [mg/ml] [MU/ml]

0 4.1 3.1 4.1 3.1 1 4.5 3.1 4.6 3.0 2 4.6 3.2 4.8 3.1 3 4.3 3.1 4.0 3.0 4 4.8 3.1 4.7 3.1 5 4.1 3.3 4.1 3.2 6 4.2 3.0 4.2 3.1 7

4.1 3.1 4.3 3.2 8 4.0 2.9 4.3 3.1 Control 4.3 3.0 4.3 3.1 Control: Untreated sample measured AA: Amidolytic activity C.sub.prot. : Protein concentration

Detailed Description Paragraph Table (3):

TABLE 2 150 mM of Na.sub.2 HPO.sub.4 /H.sub.3 PO.sub.4, pH 6.0 10 mM of TES 50 mg/ml of trehalose 0.01% of Tween 80 -20.degree. C. +4.degree. C. C.sub.prot. AA C.sub.prot. AA Day [mg/ml] [MU/ml] [mg/ml] [MU/ml] 0 3.9 2.1 7 3.9 2.3 3.97 2.4 14 3.9 2.3 3.98 2.4 20 3.9 2.3 3.9 2.4 30 3.9 2.3 3.9 2.4 AA: Amidolytic activity C.sub.prot. : Protein concentration

Detailed Description Paragraph Table (4):

TABLE 3 200 mM of Na.sub.2 HPO.sub.4 /H.sub.3 PO.sub.4, pH 6.0 10 mM of TES 50 mg/ml of saccharose 0.01% of Tween 80 -20.degree. C. +4.degree. C. C.sub.prot. AA C.sub.prot. AA Day [mg/ml] [MU/ml] [mg/ml] [MU/ml] 0 6.0 3.3 6.0 3.3 1 5.7 3.3 5.7 3.6 2 5.8 3.3 5.9 3.4 5 5.9 3.2 6.0 3.6 9 6.0 3.3 5.9 3.7 15 5.8 3.5 5.9 3.5 29 5.9 3.6 5.9 3.8 AA: Amidolytic activity C.sub.prot. : Protein concentration

Detailed Description Paragraph Table (5):

TABLE 4 150 mM of Na.sub.2 HPO.sub.4 /H.sub.3 PO.sub.4, pH 6.0 10 mM of TES 50 mg/ml of saccharose 0.01% of Tween 80 -20.degree. C. -70.degree. C. C.sub.prot. AA C.sub.prot. AA Day [mg/ml] [MU/ml] [mg/ml] [MU/ml] 0 5.9 3.5 5.9 3.5 1 5.5 3.5 5.6 3.4 2 5.6 3.4 5.5 3.5 5 6.0 3.5 6.2 3.6 9 6.0 3.3 6.0 3.3 15 6.0 3.6 5.9 3.1 29 5.9 3.5 6.0 3.6 AA: Amidolytic activity C.sub.prot. : Protein concentration

Detailed Description Paragraph Table (6):

TABLE 5 150 mM of Na.sub.2 HPO.sub.4 /H.sub.3 PO.sub.4, pH 6.0 10 mM of TES 50 mg/ml of saccharose 0.01% of Tween 80 C.sub.prot. AA Day [mg/ml] [MU/ml] 0 10.2 4.8 5 10.2 4.9 AA: Amidolytic activity C.sub.prot. : Protein concentration

Detailed Description Paragraph Table (7):

Solution A: BM 06.022 4 mg/ml Na.sub.2 HPO.sub.4.12H.sub.2 O 53.72 mg/ml H.sub.3 PO.sub.4 85% 11.3 mg/ml Tranexamic acid 1.6 mg/ml Saccharose 50 mg/ml Tween 80, pH 6 0.1 mg/ml Solution B: BM 06.022 4 mg/ml K.sub.2 HPO.sub.4 26.2 mg/ml H.sub.3 PO.sub.4 85% 11.6 mg/ml Tranexamic acid 1.6 mg/ml Saccharose 70 mg/ml Tween 80, pH 6 0.1 mg/ml

Detailed Description Paragraph Table (8):

TABLE 6 Composition of Real and Placebo Solutions 1. Real Solutions Test No. 93/1292 Test No. 93/1294 BM 06.022 10 MU 10 MU Dipotassium hydrogen 131.0 mg 131.0 mg phosphate Phosphoric acid 85% 58 mg 58 mg Tranexamic acid 8.0 mg 8.0 mg Saccharose 250.0 mg 350.0 mg Polysorbate 80 0.5 mg 0.5 mg Water p.i. q.s. 10.0 ml q.s. 10.0 ml pH 6.0 6.0 Osmolarity 320 mOsmoles 351 mOsmoles 2. Placebo Solutions Test No. 93/1291 Test No. 93/1293 Dipotassium hydrogen 131.0 mg 131.0 mg phosphate Phosphoric acid 85% 58 mg 58 mg Tranexamic acid 8.0 mg 8.0 mg Saccharose 250.0 mg 350.0 mg Polysorbate 80 0.5 mg 0.5 mg Water p.i. q.s. 10.0 ml q.s. 10.0 ml pH 6.0 6.0 Osmolarity 322 mOsmoles 343 mOsmoles

CLAIMS:

1. A pharmaceutical composition comprising a protein having plasminogen activator activity, sugar and tranexamic acid, wherein said tranexamic acid is the only aminocarboxylic acid in said composition, and wherein said composition is devoid of citrate.

2. The composition according to claim 1, wherein said sugar is a disaccharide.

9. The composition according to claim 1, wherein said sugar is present in an amount of 40-100 mg/ml.

10. The composition according to claim 7, wherein said sugar is present in an amount of 50-70 mg/ml.

11. The composition according to claim 1, wherein said tranexamic acid is present in an amount of 1-50 mmoles/l.

12. The composition according to claim 11, wherein said tranexamic acid is present in an amount of 8-12 mmoles/l.

21. A composition in the form of a lyophilisate with a residual moisture content of less than 5%, comprising a protein having plasminogen activator activity, sugar, phosphate buffer, tranexamic acid and a surfactant, wherein said composition is devoid of citrate and wherein said tranexamic acid is the only aminocarboxylic acid in said composition.

24. A process for preventing the loss of plasminogen activator activity or for preventing the formation of protein aggregates in a composition which contains a protein having plasminogen activator activity, comprising mixing said protein having plasminogen activator activity with sugar and tranexamic acid, wherein said tranexamic acid is the only aminocarboxylic acid in said composition, and wherein said composition is devoid of citrate.

25. A process for preparing a lyophilized pharmaceutical composition comprising a protein having plasminogen activator activity, sugar and tranexamic acid, wherein said tranexamic acid is the only aminocarboxylic acid in said composition, and wherein said composition is devoid of citrate, comprising

preparing an aqueous solution containing a protein having plasminogen activator activity, sugar, and tranexamic acid,

freezing the aqueous solution,

adjusting the temperature of the frozen solution to a value below the glass temperature, and

lyophilizing the frozen solution in a vacuum.

28. A method for treating blood clots comprising administering to a patient in need of such treatment, an effective amount of a composition comprising an effective amount of a protein having plasminogen activator activity, sugar and tranexamic acid, wherein said tranexamic acid is the only aminocarboxylic acid in said composition, said composition is devoid of citrate and wherein said composition is in a vein-tolerated pharmaceutical administration form.

29. A pharmaceutical composition comprising a protein having plasminogen activator activity, sugar and a single aminocarboxylic acid, wherein said single aminocarboxylic acid is tranexamic acid, and wherein said composition is devoid of citrate.

L10: Entry 39 of 40 File: USPT Aug 14, 1984

DOCUMENT-IDENTIFIER: US 4465662 A
TITLE: Oral compositions of tranexamic acid and carvone

Brief Summary Text (18):
The amount of tranexamic acid blended is not particularly limited in the present invention, but is generally in the range of 0.01 to 5% by weight of the composition. In addition to tranexamic acid, the oral composition of this invention may further include other additional active ingredients, for example, enzymes such as amylase, protease, mutanase, lysozyme, lytic enzyme, etc., fluorine compounds such as alkali metal monofluorophosphates (e.g., disodium monofluorophosphate, dipotassium monofluorophosphate, etc.) and metal fluorides (e.g., sodium fluoride, stannous fluoride, etc.), stannous compounds, chlorhexidine salts, .epsilon.-aminocaproic acid, aluminum chlorohydroxyallantoinate, dihydrocholesterol, glycyrrhetinates, glycerophosphate, sodium chloride, water-soluble inorganic phosphates (e.g., potassium and sodium salts of orthophosphoric acid, pyrophosphoric acid and polyphosphoric acid) and the like alone or in admixture.

Brief Summary Text (27):
The toothpastes may generally have a pH of 4.5 to 10, preferably 6 to 8.5.

L10: Entry 35 of 40 File: USPT Dec 3, 1985

DOCUMENT-IDENTIFIER: US 4556553 A
TITLE: Toothpaste composition

Detailed Description Text (20):
In addition to fluorides, the toothpaste composition of this invention may further include other active ingredients, for example, enzymes such as amylase, protease, mutanase, lysozyme, lytic enzyme, etc., stannous compounds, .epsilon.-aminocaproic acid, tranexamic acid, aluminum chlorohydroxyallantoinate, dihydrocholesterol, glycyrrhetinates, glycerophosphate, sodium chloride, water-soluble inorganic phosphates, and the like alone or in admixture. Examples of the watersoluble inorganic phosphate are potassium and sodium salts of orthophosphoric acid, pyrophosphoric acid and polyphosphoric acid, while the potassium salts are preferred.

Detailed Description Text (24):
The toothpaste composition according to this invention may generally have a pH or 4.5 to 10, preferably 6 to 8.5.

CLAIMS:

9. A toothpaste composition according to claim 1 wherein the pH of the toothpaste composition is 4.5 to 10.

20. A toothpaste composition according to claim 10 wherein the pH of the toothpaste composition is 4.5 to 10.

L10: Entry 34 of 40 File: USPT Mar 18, 1986

DOCUMENT-IDENTIFIER: US 4576816 A
TITLE: Dentifrice composition

Brief Summary Text (25):
In addition to dextranase, the dentifrice composition of this invention may further include other additional active ingredients, for example, enzymes such as amylase, protease, mutanase, lysozyme, lytic enzyme, etc., alkali metal monofluorophosphates such as sodium monofluorophosphate and potassium monofluorophosphate, fluorides such as sodium fluoride, stannous fluoride, etc., chlorohexidine salts, .epsilon.-aminocaproic acid, tranexamic acid, aluminum chlorohydroxyallantoinate, dihydrocholesterol, glycyrrhetinates, glycerophosphate, sodium chloride, water-soluble inorganic phosphates, and the like alone or in admixture. Preferably alkali metal monofluorophosphates such as sodium monofluorophosphate may be combined with dextranase because they not only stabilize dextranase, but also retain sufficient dextranase in aged dentifrice compositions. In this case, the content of alkali metal monofluorophosphates may preferably be in the range of 0.1-1% by weight. Mutanase coacts with dextranase to provide a synergistic effect of dissolving dental plaque and preventing reformation of dental plaque. Dextranase may advantageously be combined with lytic enzyme to increase the efficacy thereof. Examples of the water-soluble inorganic phosphate are potassium and sodium salts of orthophosphoric acid, pyrophosphoric acid and polyphosphoric acid, while the potassium salts are preferred.

Brief Summary Text (30):
The toothpaste composition according to this invention may generally have a pH of 4.5 to 10, preferably 6 to 8.5.

CLAIMS:

5. A dentifrice composition according to claim 2, having a pH of 4.5 to 10.

6. A dentifrice composition according to claim 2, having a pH of 6 to 8.5.

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L7: Entry 9 of 12 File: USPT Dec 22, 1998

DOCUMENT-IDENTIFIER: US 5851836 A
TITLE: Method for determining fibrinogen and reagent for determination thereof

Abstract Text (1):
A method for determining fibrinogen concentration whereby thrombin or a protease inhibitor having similar activity thereto is added to an undiluted to test sample to convert fibrinogen in the sample to fibrin, and determining a coagulation time fibrinogen is converted in a reaction mixture containing a salt at a high concentration and a reagent therefor. The determination in the presence of a salt at a high concentration permits simplified determination without diluting the test sample.

Brief Summary Text (19):
The present invention provides a method for determining fibrinogen in a test sample, comprising adding thrombin or a protease having a similar activity thereto to a test sample to convert fibrinogen in the sample to fibrin, and determining a coagulation time, wherein an undiluted sample is used and the fibrinogen is converted in a reaction mixture containing a salt at a high concentration.

Brief Summary Text (20):
The present invention also relates to a reagent for fibrinogen determination, comprising a salt at a high concentration, and 20-500 NIHU/ml thrombin or a protease having a similar activity.

Detailed Description Text (3):
According to the method of the present invention, fibrinogen in a sample is converted to fibrin by the action of thrombin or a protease having a similar activity. The coagulation time varies depending on the fibrinogen concentration, and when the determination conditions are the same, the coagulation time corresponds to the fibrinogen concentration. Comparison thereof with the coagulation time of the standard leads to the determination of fibrinogen.

Detailed Description Text (5):
The origin of the thrombin to be used in the present invention is not particularly limited, and thrombins derived from human, cow, horse, goat and the like can be used, and they are commercially available. The "similar activity" in "a protease having a similar activity" means the protease activity which forms fibrin by acting on fibrinogen. The origin of such protease is not particularly limited, and batroxobin, a protease derived from venom of a snake (academic designation: Agkistrodon rhodostoma) and the like can be used.

Detailed Description Text (6):
When thrombin is used in the present invention, an excess amount of at least 20 NIHU is added per 1 ml of plasma. When a protease having a similar activity is used, an amount thereof is added, which shows a similar activity when converted to thrombin. The concentration of thrombin or a protease having a similar activity in a reaction mixture is generally 10-200 NIHU/ml, preferably 20-100 NIHU/ml.

Detailed Description Text (7):
In the method of the present invention, a sample and a reagent containing thrombin or a protease having a similar activity are mixed at a volume ratio of 1:1-1:8, preferably 1:2-1:3.

Detailed Description Text (20):
The fibrinogen determination reagent of the present invention contains a salt at a high concentration and 20-500 NIHU/ml thrombin or a protease having a similar activity.

Detailed Description Text (21):
The fibrinogen determination reagent of the present invention consists of one or two kinds of constituent reagents (hereinafter referred to simply as constituents), with preference given to one kind reagent. When a discrepancy preventive is used, the reagent preferably consists of two kinds of constituents, that is, a first reagent containing a discrepancy preventive and a second reagent containing thrombin or a protease having a similar activity. When the reagent of the present invention contains two kinds of constituents, a protease such as thrombin is contained in a second reagent, and a salt is contained in either or both of a first reagent and a second reagent. When the reagent of the present invention contains one kind of constituent, it contains 20-500 NIHU/ml, preferably 40-200 NIHU/ml thrombin, 10-400 mM, preferably 30-200 mM buffer (pH 6.0-9.0, preferably 7.0-8.0) and a salt at a high concentration.

Detailed Description Text (27):
In the present invention, various additives can be added on demand for the purpose of maintaining the property and quality of the reagent, production and the like. Examples of the additive include proteins such as BSA, chelating agents such as ethylenediaminetetraacetic acid (EDTA) and ethylene glycol bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), antifibrinolysis such as .epsilon.-aminocaproic acid, tranexamic acid and aprotinine, heparin inhibitors such as polyburene, preservatives such as sodium azide and gentamycin sulfate, surfactants such as polyoxyethylene glycol p-t-octylphenyl ethers (e.g. Triton X-100, trademark) and polyoxyethylene glycol sorbitan alkyl ethers (e.g. Tween 20, trademark), and substances such as sugars (e.g. lactose), amino acids, polyethylene glycol and glycerol.

CLAIMS:

1. A method for determining a fibrinogen concentration, consisting essentially of adding thrombin or a protease having a similar activity thereto to an undiluted test sample to convert fibrinogen in the sample to fibrin, and determining a coagulation time as a measure of fibrinogen concentration, wherein the conversion of the fibrinogen to fibrin is carried out in a solution containing at least one salt selected from the group consisting of sodium salt, potassium salt and magnesium salt at a concentration, said concentration of the salt being set for a level which provides a coagulation time of 5-100 seconds when the coagulation time is measured at 37.degree. C. using a mixture of a sample containing fibrinogen (275 mg/dl), and a reagent containing thrombin (100 NIHU/ml) and HEPES (100 mM, pH 7-35), at a volume ratio of the sample to the reagent of 1:2.

2. The method of claim 1, wherein a sample and a reagent containing thrombin or a protease having a similar activity thereto are mixed at a volume ratio of 1:1-1:8.

13. A reagent for determining a fibrinogen quantity consisting essentially of, a salt selected from the group consisting of sodium salt, potassium salt, and magnesium salt, and 20-500 NIHU/ml thrombin or a protease having a similar activity thereto, wherein the concentration of the salt is set for a level which provides a coagulation time of 5-100 seconds when the coagulation time is measured at 37.degree. C. using a mixture of a sample containing fibrinogen (275 mg/dl), and a reagent containing thrombin (100 NIHU/ml) and HEPES (100 mM, pH 7.35) at a volume ratio of the sample and the reagent of 1:2.

22. The reagent of Claim 21, comprising a first reagent comprising the discrepancy preventive, and a second reagent comprising thrombin or a protease having a similar activity thereto, wherein the salt is comprised in either or both of the first reagent and the second reagent.

L7: Entry 33 of 78 File: USPT Mar 8, 1994

DOCUMENT-IDENTIFIER: US 5292528 A
TITLE: Oral Composition

Detailed Description Text (29):
Examples of the therapeutic agent are cationic bactericides such as cetylpyridium chloride and chlorhexidine salts; nonionic bactericides such as tricrosan; amphoteric bactericides such as dodecyldiaminoethylglycine; enzymes such as dextranase, amylase, protease, mutanase, lysozyme and lytic enzymes; monofluorophosphates of alkali metals, such as sodium monofluorophosphate and potassium monofluorophosphate; fluorides such as sodium fluoride and stannous fluoride; tranexamic acid and .epsilon.-aminocapric acid; aluminum chlorhydroxyl allantoin; dihydrocholesterol, glycyrrhizin salts, glycyrrhetic acid, glycerophosphate, chlorophyll, sodium chloride, caropeptide and water-soluble compounds of inorganic phosphoric acid. These therapeutic agents may be used alone or in combination.

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L7: Entry 12 of 12 File: USPT Nov 26, 1991

DOCUMENT-IDENTIFIER: US 5068106 A
TITLE: t-PA solution of high concentration and use of the solution in human and veterinary medicine

Abstract Text (2):
Hence, the present invention relates to a process for the preparation of a solution of high concentration of a protein having plasminogen activator activity, where an increase in stability and solubility is achieved by adding at least two substances from the group of D- and/or L-amino acids, their salts, derivatives or homologs. This invention also relates to a process for the pasteurization of a protein solution having t-PA activity, and to a t-PA-containing solution prepared by the claimed process, and to the use of this solution as a fibrinolytic in human and veterinary medicine.

Brief Summary Text (4):
Plasmin, which has thrombolytic activity, is a relatively non-specific, trypsin-like serine protease. It is synthesized in the form of an inactive precursor, plasminogen. Plasminogen circulates as inactive precursor in the blood and is activated only in response to particular stimuli. The conversion of plasminogen into plasmin is catalysed by plasminogen activators.

Brief Summary Text (13):
t-PA concentrations of 3,000 to 50,000 U/ml can be reached by addition of lysine or ornithine under the conditions described in EP-A 156,169. This would result, at the therapeutic dosage required, in delivery of a very large amount of liquid. Addition of lysine or ornithine as described in European Patent Application EP 156,169 cannot result in solutions containing t-PA which can be reasonably used therapeutically.

Brief Summary Text (16):
This object is achieved according to the invention by adding to the solution at least two substances from the group of D- and/or L-amino acids, their salts, derivatives or homologs.

Brief Summary Text (17):
It has emerged, surprisingly, that addition of at least two substances from the group of D- and/or L-amino acids, their salts, derivatives or homologs has a stability-promoting effect on proteins having t-PA activity. At the same time, an increased solubility of the protein can be observed by combination of several of these substances. The extent of both effects is completely unexpected. Both the increase in stability and the rise in the solubility are apparently derived from a synergism of the individual actions.

Detailed Description Text (1):
The substances according to the invention, for example lysine, ornithine, arginine, diaminopimelic acid, agmatine, creatine, guanidinoacetic acid, acetylornithine, citrulline, argininosuccinic acid, tranexamic acid and c-aminocaproic acid, are outstandingly suitable for the formulation of solutions intended for infusion. A feature which is common to them all is the presence of a basic group in the form of an amino and/or a guanidino group.

Detailed Description Text (2):

A combination of arginine and lysine, preferably 0.001 to 1 mol/1, particularly preferably 0.01 to 0.5 mol/1, has proved particularly suitable for the preparation of a solution of high concentration of a protein having plasminogen activator activity. Thus, for example, it has been shown that the t-PA activity in a cell culture supernatant had fallen to 31% of the initial value after incubation at +4.degree. C. for 5 days, whereas addition of 0.1 mol/1 arginine and 0.1 mol/1 lysine to a parallel sample resulted in a decrease in activity of only 5% after the same incubation time.

Detailed Description Text (4):
The isolation of the t-PA or of a protein having the same activity can be carried out by customary methods. Where appropriate, the protein having t-PA activity is first dissolved by dialysis against a buffered solution of a chaotropic agent, for example KSCN, concentrated in this state to the desired t-PA concentration, and then dialyzed against a buffered solution containing at least two of the substances according to the invention, preferably arginine and lysine. For example, this will entail the solution of a protein having plasminogen activator activity being dialyzed first against approximately 1.6 mol/1 KSCN in approximately 0.05 mol/1 tris-HCl, pH 7, and then against arginine and lysine, each 0.1 mol/1, in 0.05 mol/1 tris-HCl, pH 7.

Detailed Description Text (7):
It is customary for solutions intended for parenteral administration to be sterilized by filtration because many of the biologically active molecules would be decomposed by pasteurization. However, the presence of viruses can never be completely ruled out in products sterilized by filtration, as is demonstrated by the SV 40 contamination of smallpox vaccine or the transmission of the AIDS virus by factor VIII preparations. A considerable advantage of the process which is described here is that the proteins having t-PA activity are sufficiently stabilized by the addition of substances according to the invention that their activity is substantially retained even after pasteurization. Whereas a buffered t-PA-containing solution had, for example, after a low-temperature pasteurization, retained only 0.5 per cent of the initial activity, it was possible to retain about 85 per cent of the initial activity by addition of arginine and lysine, 1 mol/1 each.

Detailed Description Text (11):
The protein-containing solution can, where appropriate, be mixed with further additives, for example mono- or disaccharides, sugar alcohols and, where appropriate, other additional components such as, for example, albumins, gelatin, Haemaccel, sodium chloride, calcium chloride, heparin, EDTA, glycine or detergents. These additional components are added in physiologically tolerated amounts for the purpose of, for example, stabilization, buffering of the system, inhibition of proteases, reducing the surface tension and regulating the osmolarity.

Detailed Description Text (12):
Addition of sucrose or sorbitol has a further stabilitypromoting effect during the pasteurization. Thus, the content of active molecules remaining after pasteurization in a glycine-buffered t-PA solution which contains arginine and lysine, 0.5 mol/1 each, can be increased from about 81 per cent to 96 per cent. In this connection, addition of 0.2 to 2 kg of sucrose or sorbitol per liter is preferred.

Detailed Description Text (21):
The cell culture supernatants harvested under sterile conditions were stored at +4.degree. C. for 5 days in the presence or absence of 0.1 mol/1 arginine and 0.1 mol/1 lysine.

Detailed Description Text (24):
However, the decrease in activity of the cell culture supernatant treated according to the invention is only 5 per cent, so that addition of arginine and lysine makes it possible for cell culture supernatants to be stored temporarily without considerable loss of activity.

Detailed Description Text (26):
A t-PA-containing solution was dialyzed against 1.6 mol/1 KSCN in 0.05 mol/1 tris-HCl, pH 7.0, and then against 0.1 mol/1 arginine and 0.1 mol/1 lysine in 0.05

mol/l tris-HCl, pH 7.0, and was concentrated to 0.1, 0.2, 0.5, 1.0, 10, 20, 40, 60 and 80 mg of t-PA/ml. It was observed that it was possible to keep t-PA in solution up to a concentration of 60 mg/ml. The solubility limit with Tween 80.sup.(.RTM.) (0.1%) without other additives was 0.5 mg of t-PA/ml.

Detailed Description Text (28):
A t-PA-containing solution was dialyzed against 1.6 mol/l KSCN in 0.05 mol/l tris, pH 7.0, and then against a buffer containing 0.05 mol/l glycine, pH 7.0, together with increasing concentrations of arginine and lysine. 0.5 or 1 g/ml sucrose was added to the t-PA-containing solution where indicated. The pH of the solutions was adjusted to 7. The solutions were heated at 60.degree. C. for 10 h. The t-PA activity was determined before and after pasteurization.

Detailed Description Text (29):
The table shows that both sucrose and the addition of arginine and lysine have a stabilizing effect on plasminogen activator. Whereas the activity in a sample to which neither sucrose nor arginine or lysine has been added is almost completely destroyed by pasteurization, after addition merely of one gram of sucrose/ml 68 per cent of the initial activity is retained. This figure can be considerably improved by addition of arginine and lysine, so that 96 per cent of the initial activity is retained with a combination of 1 g/ml sucrose and of arginine and lysine, 0.5 mol/l each, which means that the loss of activity due to pasteurization becomes negligibly small.

Detailed Description Paragraph Table (2):

Concentration of Activity after pasteurization (%) arginine + lysine			Sucrose concentration (g/ml)			(mol/l)			0			0.5			1
--	--	0.5	--	68	0.001	0.001	56.0	--	--	0.05	0.05	56.9	--	--	0.1
0.1	71.3	88.0	88.0	0.2	0.2	76.1	--	--	0.5	0.5	80.7	91.0	96.3	1.0	1.0
85.6	--	--													

CLAIMS:

1. A process for the preparation of a protein solution having tissue plasminogen activator (t-PA) activity, comprising

preparing a solution containing t-PA in the one- or two-chain form, or a solution containing a derivative of said t-PA which occurs naturally or which has been prepared by synthesis or genetic manipulation; and

adding to said solution 0.001 to 1 mol/l of arginine and 0.001 to 1 mol/l of lysine.

3. The process as claimed in claim 1, wherein said addition step comprises dialyzing said solution containing t-PA against a buffered solution containing arginine and lysine.

6. The process as claimed in claim 5, further comprising, after adding the lysine and arginine and before the pasteurization step, adding an effective amount of a pasteurization stabilizing substance selected from the group consisting of sucrose and sorbitol.

8. The process as claimed in claim 1, wherein 0.01 to 0.5 mol/l of arginine and lysine are added.

11. The process as claimed in claim 3, wherein said buffer solution contains 0.1 mol/l arginine and 0.1 mol/l lysine, and has a pH of 7.

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L7: Entry 3 of 12 File: USPT Jan 15, 2002

DOCUMENT-IDENTIFIER: US 6338855 B1
TITLE: Cleansing articles for skin and/or hair which also deposit skin care actives

Brief Summary Text (76):
Alkyl glucosides and alkyl polyglucosides are useful herein, and can be broadly defined as condensation articles of long chain alcohols, e.g. C8-30 alcohols, with sugars or starches or sugar or starch polymers, i.e., glycosides or polyglycosides. These compounds can be represented by the formula (S).sub.n --O--R wherein S is a sugar moiety such as glucose, fructose, mannose, and galactose; n is an integer of from about 1 to about 1000, and R is a C8-30 alkyl group. Examples of long chain alcohols from which the alkyl group can be derived include decyl alcohol, cetyl alcohol, stearyl alcohol, lauryl alcohol, myristyl alcohol, oleyl alcohol, and the like. Preferred examples of these surfactants include those wherein S is a glucose moiety, R is a C8-20 alkyl group, and n is an integer of from about 1 to about 9. Commercially available examples of these surfactants include decyl polyglucoside (available as APG 325 CS from Henkel) and lauryl polyglucoside (available as APG 600CS and 625 CS from Henkel). Also useful are sucrose ester surfactants such as sucrose cocoate and sucrose laurate.

Brief Summary Text (78):
wherein: R.sup.1 is H, C.sub.1 -C.sub.4 alkyl, 2-hydroxyethyl, 2-hydroxy-propyl, preferably C.sub.1 -C.sub.4 alkyl, more preferably methyl or ethyl, most preferably methyl; R is C.sub.5 -C.sub.31 alkyl or alkenyl, preferably C.sub.7 -C.sub.19 alkyl or alkenyl, more preferably C.sub.9 -C.sub.17 alkyl or alkenyl, most preferably C.sub.1 -C.sub.15 alkyl or alkenyl; and Z is a polhydroxyhydrocarbyl moiety having a linear hydrocarbyl chain with a least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative (preferably ethoxylated or propoxylated) thereof. Z preferably is a sugar moiety selected from the group consisting of glucose, fructose, maltose, lactose, galactose, mannose, xylose, and mixtures thereof. An especially preferred surfactant corresponding to the above structure is coconut alkyl N-methyl glucoside amide (i.e., wherein the R.sup.2 CO-- moiety is derived from coconut oil fatty acids). Processes for making compositions containing polyhydroxy fatty acid amides are disclosed, for example, in G. B. Pat. Specification 809,060, published Feb. 18, 1959, by Thomas Hedley & Co., Ltd.; U.S. Pat. No. 2,965,576, to E. R. Wilson, issued Dec. 20, 1960; U.S. Pat. No. 2,703,798, to A. M. Schwartz, issued Mar. 8, 1955; and U.S. Pat. No. 1,985,424, to Piggott issued Dec. 25, 1934; which are incorporated herein by reference in their entirety.

Brief Summary Text (80):
Nonlimiting examples of preferred nonionic surfactants for use herein are those selected form the group consisting of C8-C14 glucose amides, C8-C14 alkyl polyglucosides, sucrose cocoate, sucrose laurate, lauramine oxide, cocoamine oxide, and mixtures thereof.

Brief Summary Text (122):
Nonlimiting examples of useful anti-acne actives include the keratolytics such as salicylic acid (o-hydroxybenzoic acid), derivatives of salicylic acid such as 5-octanoyl salicylic acid and 4 methoxysalicylic acid, and resorcinol; retinoids such as retinoic acid and its derivatives (e.g., cis and trans); sulfur-containing D and L amino acids and their derivatives and salts, particularly their N-acetyl derivatives, a preferred example of which is N-acetyl-L-cysteine; lipoic acid; antibiotics and antimicrobials such as benzoyl peroxide, octopirox, tetracycline,

2,4,4'-trichloro-2'-hydroxy diphenyl ether, 3,4,4'-trichlorobanilide, azelaic acid and its derivatives, phenoxyethanol, phenoxypropanol, phenoxyisopropanol, ethyl acetate, clindamycin and meclocycline; sebostats such as flavonoids and bioflavonoids; bile salts such as scymnol sulfate and its derivatives, deoxycholate, and cholate; abietic acid; adapalene; allantoin; aloe extracts; arbietic acid and its salts; aryl-2,4 dioxo oxazolidine derivatives; ASEBIOL (available from Laboratories Serobiologiques, located in Somerville, N.J.); azaleic acid; barberry extracts; bearberry extracts; belamcanda chinensis; benzoquinolinones; benzoyl peroxide; berberine; BIODERMINE (available from Sederma, located in Brooklyn, N.Y.); bioflavinoids; bisabolol; S-carboxymethyl cysteine; carrot extracts; cassin oil; clove extracts; citral; citronellal; climazole; Completech MBAC-OS (available from Lipo); CREMOGEN M82 (available from Dragoco, located in Totowa, N.J.); cucumber extracts; dehydroacetic acid and its salts; dehydroeplandersterone salicylate; dichlorophenyl imidazoldioxolan which is commercially available as COMPLETECH MBAC-OS (from Lipo, located in Paterson, N.J.); DL valine and its esters; DMDM hydantoin; Epicutin TT (available from CLR); erythromycin; escinol; ethyl hexyl monoglyceryl ether; ethyl 2-hydroxy undecanoate; farnesol; farnesol acetate; geranoil; glabridin; gluconic acid; gluconolactone; glyceryl monocaprate; glycolic acid; grapefruit seed extract; gugu lipid; Hederagenin (available from Maruzen); hesperitin; hinokitol; hops extract; hydrogenated rosin; 10 hydroxy decanoic acid; ichtyhol; interleukin 1 alpha antagonists; iodo-2-propynyl butyl carbamate; Kapilarine (available from Greentech); ketoconazole; lactic acid; lemon grass oil; Lichochalcone LR15 (available from Maruzen); linoleic acid; LIPACIDE C8CO (available from Seppic, located in Paris, France); lovastatin; 4 methoxysalicylic acid; metronidazole; minocycline; mukurossi; neem seed oil; vitamin B.sub.3 compounds (such as niacinamide and nicotinic acid); nisin; 5-octanoly salicylic acid; octopirox; panthenol; 1-pentadecanol; peonia extract; peppermint extract; phelladendron extract; 2-phenyl-benzothiophene derivatives; phloretin; PHLOROGINE (available from Secma); phosphatidyl choline; proteolytic enzymes; quercetin; red sandalwood extract; resorcinol; rosemary extract; rutin; sage extract; salicin; salicylic acid; skull cap extract; siber hegner extract; siberian saxifrage extract; silicol; sodium lauryl sulfate; sodium sulfoacetamide; Sophora Extract (available from Maruzen); sorbic acid; sulfur; sunder vati extract; tea tree oil; tetracyline; tetra hydroabietic acid; thyme extract; tioxolone; tocopherol; trehalose 6-undecylenoate; 3 tridecene-2-ol; triclosan; tropolone; UNITRIENOL T27 (available from Unichem, located in Gouda, Netherlands); vitamin D.sub.3 and its analogs; white thyme oil; willow bark extract; wogonin; Ylang Ylang; zinc glycerolate; zinc linoleate; zinc oxide; zinc pyrithione; zinc sulfate and mixtures thereof

Brief Summary Text (126):

Anti-wrinkle, anti-skin atrophy and skin repair actives can be effective in replenishing or rejuvenating the epidermal layer. These actives generally provide these desirable skin care benefits by promoting or maintaining the natural process of desquamation. Nonlimiting examples of antiwrinkle and anti-skin atrophy actives include retinoic acid and its derivatives (e.g., cis and trans); retinal; retinol; retinyl esters such as retinyl acetate, retinyl palmitate, and retinyl propionate; vitamin B.sub.3 compounds (such as niacinamide and nicotinic acid), salicylic acid and derivatives thereof (such as 5-octanoyl salicylic acid, heptyloxy 4 salicylic acid, and 4-methoxy salicylic acid); sulfur-containing D and L amino acids and their derivatives and salts, particularly the N-acetyl derivatives, a preferred example of which is N-acetyl-L-cysteine; thiols, e.g. ethane thiol; hydroxy acids, phytic acid, lipoic acid; lysophosphatidic acid; skin peel agents (e.g., phenol and the like); Actein 27-Deoxyactein Cimicifugoside (available from Cirnigoside); adapalene; ademethionine; adenosine; aletris extract; alkyl glutathione esters; alkoxyalkoxy alkoxyn benzoic and derivatives; aloe derived lectins; amino propane phosphoric acid; 3-aminopropyl dihydrogen phosphate; Amadorine (available from Barnet Products); anise extracts; AOSINE (available from Secma); arginine amino benzoate; ASC III (available from E. Merck, located in Darmstadt, Germany); ascorbic acid; ascorbyl palmitate; asiatic acid; asiaticosides; ARLAMOL GEO.TM. (available from ICI, located in Wilmington, Del.); azaleic acid; benzoic acid derivatives; bertholletia extracts; betulinic acid; BIOCHANIN A AND BIOPEPTIDE CL (available from Sederma, located in Brooklyn, N.Y.); BIOPEPTIDE EL (available from Sederma); biotin; blackberry bark extract; blackberry lily extracts; black cohosh extract; blue cohesh extract; butanoyl betulinic acid; carboxymethyl 1,3 beta glucan; catecholamnines; chalcones; citric acid esters; chaste tree extract; clover extracts; coumestrol; CPC

Peptide (available from Barnet Products); daidzein; dang gui extract; darutoside; debromo laurinterol; 1-decanoyl-glycero-phosphonic acid; dehydrocholesterol; dehydrodicreosol; dehydrodieugenol; dehydroepiandersterone; DERMOLECTINE (available from Sederma); dehydroascorbic acid; dehydroepiandersterone sulfate; dianethole; dihydroxy benzoic acid; 2,4 dihydroxybenzoic acid; diglycol guanidine succinate; diosgenin; disodium ascorbyl phosphate; dodecanedioic acid; Ederline (available from Seporga); Enderline (available from Laboratories Seporga); equol; eriodictyol; estrogen and its derivatives; ETF (available from Laboratories Seporga); ethocyn; ELESERYL SH (available from Laboratories Serobiologiques, located in Somerville, N.J.); ENDONUCLEINE (available from Laboratories Serobiologiques); ergosterol; eythrobic acid; fennel extract; fenugreek seed extract; FIBRASTIL (available from Sederma); FIBROSTIMULINES S and P (available from Sederma); FIRMOGEN LS 8445 (available from Laboratories Serobiologiques); formononetin; forsythia fruit extract; gallic acid esters; gamma amino butyric acid; GATULINE RC (available from Gattlefosse, located in Priest, France); genistein; genisteine; genistic acid; gentisyl alcohol; gingko bilboa extracts; ginseng extracts; ginsenoside (RO, R.sub.6-1, R.sub.6-2, R.sub.6-3, R.sub.C, R.sub.D, R.sub.E, R.sub.F, R.sub.F-2, R.sub.G-1, R.sub.G-2); gluco pyranosyl-L-ascorbate; glutathione and its esters; glycitein; hesperitin; hexahydro curcumin; HMG- coenzyme A reductase inhibitors; hops extracts; 11 hydroxy undecanoic acid; 10 hydroxy decanoic acid; 25-hydroxycholesterol; 7-hydroxylated sterols; hydroxyethyl isostearyloxy isopropanolamine; hydroxy-tetra methyl piperidinyloxy; hypotaurine; ibukijakou extract; isoflavone SG 10 (available from Barnet Products); kinetin; kohki extract; L-2-OXO-thiazolidine-4-carboxylic acid esters; lactate dehydrogenase inhibitors; 1-lauryl, -lyso-phosphatidyl choline; lectins; lichochalcone LF15 (available from Maruzen); licorice extracts; lignan; lumisterol; lupenes; luteolin; lysophosphitidic acid; magnesium ascorbyl phosphate; margin; melatonin; melibiose; metalloproteinase inhibitors; methoprene; methoprenic acid; mevalonic acid; MPC COMPLEX (available from CLR); N methyl serine; N methyl taurine; N, N.sup.1 -bis (lactyl) cysteamine; naringenin; neotigogenin; o-desmethylangoiensin; oat beta glucan; oleanolic acid; pantethine; phenylalanine; photoanethone; piperdine; placental extracts; pratensein; pregnenolone; pregnenolone acetate; pregnenolone succinate; premarin; quillaic acid; raloxifene; REPAIR FACTOR 1 and REPAIR FACTOR FCP (both available from Sederna); retinoates (esters of C.sub.2 -C.sub.20 alcohols); retinyl glucuronate; retinyl linoleate; S-carboxymethyl cysteine; SEANAMINE FP (available from Laboratories Serobiologiques); sodium ascorbyl phosphate; soya extracts; spleen extracts; tachysterol; taurine; tazarotene; tempol; thymulen; thymus extracts; thyroid hormones; tigogenin; tocopheryl retinoate; toxifolin; traumatic acid; tricholine citrate; trifoside; uracil derivatives; ursolic acid; vitamin D.sub.3 and its analogs; vitamin K; vitex extract; yam extract; yamogenin; zeatin; and mixtures thereof.

Brief Summary Text (128):

Skin barrier repair actives are those skin care actives which can help repair and replenish the natural moisture barrier function of the epidermis. Nonlimiting examples of skin barrier repair actives include Alpha Lipid (available from Lucas Meyer); ascorbic acid; biotin; biotin esters; brassicasterol; caffeine; campesterol; canola derived sterols; Cennamides (available from Ennagram); Ceramax (available from Alban Muller); CERAMAX (available from Quest, located in Ashford, England); CERAMIDE 2 and CERAMIDE HO3.TM. (both available from Sederma); CERAMIDE II (available from Quest); CERAMIDE III and IIIB (both available from Cosmoferm, located in Deft, Netherlands); CERAMIDE LS 3773 (available from Laboratories Serobiologiques); CERAMINOL (available from Inocosm); Cerasol and Cephalip (both available from Pentapharm); cholesterol; cholesterol hydroxystearate; cholesterol isostearate; 7 dehydrocholesterol; DERMATEIN BRC and DERMATEIN GSL (both available from Hormel); ELDEW CL 301 AND ELDEW PS 203 (both available from Ajinomoto); Fitobroside (available from Pentapharm); galactocerebrosides; Generol 122 (available from Henkel); glyceryl serine amide; hydroxyethyl isostearyl isopropanolamine; lactic acid; Lactomide (available from Pentapharm); lanolin; lanolin alcohols; lanosterol; lauric acid N laurylglucamide; lipoic acid; N-acetyl cysteine; N-acetyl-L-serine; N-methyl-L-Serine; Net Sterol-ISO (available from Barnet Products); vitamin B3 compounds (such as niacinamide and nicotinic acid); palmitic acid; panthenol; panthetine; phosphodiesterase inhibitors; PHYTO/CER (available from Intergen); phytoglycolipid millet extract (available from Barnet Products Distributer, located in Englewood, N.J.); PHYTOSPHINGOSINE (available from Gist

Brocades, located in King of Prussia, Pa.); PSENDOFILAGGRIN (available from Brooks Industries, located in South Plainfield, N.J.); QUESTAMIDE H (available from Quest); serine; sigmasterol; sitosterol; soybean derived sterols; sphingosine; sphingomylinase; S-lactoyl glutathione; stearic acid; Structurine (available from Silah); SUPER STEROL ESTERS (available from Croda); thioctic acid; THSC CERAMIDE OIL (available from Campo Research); trimethyl glycine; tocopheryl nicotinate; vitamin D.sub.3 ; Y2 (available from Ocean Pharmaceutical); and mixtures thereof.

Brief Summary Text (130):
Cosmetic soothing actives can be effective in preventing or treating inflammation of the skin. The soothing active enhances the skin appearance benefits of the present invention, e.g., such agents contribute to a more uniform and acceptable skin tone or color. The exact amount of anti-inflammatory agent to be used in the compositions will depend on the particular anti-inflammatory agent utilized since such agents vary widely in potency. Nonlimiting examples of cosmetic soothing agents include the following categories: propionic acid derivatives; acetic acid derivatives; fenamic acid derivatives; biphenylcarboxylic acid derivatives; and oxicams. All of these cosmetic soothing actives are fully descnbed in U.S. Pat. No. 4,985,459 to Sunshine et al., issued Jan. 15, 1991, incorporated by reference herein in its entirety. Nonlimiting examples of useful cosmetic soothing actives include acetyl salicylic acid, ibuprofen, naproxen, benoxaprofen, flurbiprofen, fenoprofen, fenbufen, ketoprofen, indoprofen, pirprofen, carprofen, oxaprozin, pranoprofen, miroprofen, tioxaprofen, suprofen, alminoprofen, tiaprofenic acid, fluprofen, bucloxic acid, absinthium, acacia, aescin, alder buckthorn extract, allantoin, aloe, APT (available from Centerchem), arnica, astragalus, astragalus root extract, azulene, Baicalin SR 15 (available from Barnet Products Dist.), baikal skullcap, baizhu, balsam canada, bee pollen, BIOPHYTEX (available from Laboratories Serobiologiques), bisabolol, black cohosh, black cohosh extract blue cohosh, blue cohosh extract, boneset, borage, borage oil, bradykinin antagonists, bromelain, calendula, calendula extract, Canadian Willowbark Extract (available from Fytokem), candelilla wax, Cangzhu, canola phytosterols, capsicum, carboxypeptidase, celery seed, celery stem extract, CENTAURIUM (available from Sederma), centaury extract, chamazulene, chamomile, chamomile extract, chaparral, chaste tree, chaste tree extract, chickweed, chicory root, chicory root extract, chirata, chishao, collodial oatmeal, comfrey, comfrey extract, CROMOIST CM GLUCAN (available from Croda), darutoside, dehurian angelica, devil's claw, divalent metals (such as, magnesium, strontium, and manganese), doggrass, dogwood, Eashave (available from Pentapharm), eleuthero, ELHIBIN (available from Pentapharm), ENTELINE 2 (available from Secma), ephedra, epimedium, esculoside; ethacrynic acid, evening primrose, eyebright, Extract LE-100 (available from Sino Lion), Fangfeng, feverfew, ficin, forsythia fruit, Fytosterol 85 (available from Fytokem), ganoderma, gaoben, Gatuline A (available from Gattefosse), gentian, germanium extract, gingko bilboa extract, ginkgo, ginseng extract, goldenseal, gorgonian extract, gotu kola, grape fruit extract, guaiac wood oil, guggal extract, helenalin esters, henna, honeysuckle flower, horehound extract, horsechestnut, horsetail, huzhang, hypericum, ichthyol, immortelle, ipecac, job's tears, jujube, kola extract, LANACHRYS 28 (available from Lana Tech), lemon oil, lianqiao, licorice root, ligusticum, ligustrum, lovage root, luffa, mace, magnolia flower, manjistha extract, margaspidin, matricin, melatonin, MICROAT IRC (available from Nurture), mints, mistletoe, Modulene (available from Seporga), mono or diglucosides of glabridin, mono or diglucosides of gentisin, MTA (5'-deoxy-5'-methythioadenosine), mung bean extract, musk, N-methyl arginine, oat beta glucan, oat extract, orange, panthenol, papain, phenoxyacetic acid, peony bark, peony root, Phytoplenolin (available from Bio Botanica), phytosphingosine, Preregen (available from Pentapharm), purslane, QUENCH T (available from Centerchem), quillaia, red sage, rehmannia, rhubarb, rosemary, rosmarinic acid, royal jelly, rue, rutin, sandlewood, sanqi, sarsaparilla, saw palmetto, SENSILINE (available from Silab), SIEGESBECKIA (available from Sederma), stearyl glycyrrhetinate, Stimutex (available from Pentapharm), storax, strontium nitrate, sweet birch oil, sweet woodruff, tagetes, tea extract, thyme extract, tienchi ginseng, tocopherol, tocopheryl acetate, triclosan, turmeric, urimei, ursolic acid, white pine bark, witch hazel xinyi, yarrow, yeast extract, yucca, and mixtures thereof.

Brief Summary Text (132):
Artificial tanning actives can help in simulating a natural suntan by increasing melanin in the skin or by producing the appearance of increased melanin in the skin.

Nonlimiting examples of artificial tanning agents and accelerators include dihydroxyacetaone; tyrosine; tyrosine esters such as ethyl tyrosinate and glucose tyrosinate; acetyl tyrosine; phospho-DOPA; brazilin; caffeine; coffee extracts; dihydroxyacetone; DNA fragments; isobutyl methyl xanthine; methyl xanthine; Phototan (available from Laboratoires Serobiologiques); prostaglandins; tea extracts; theophylline; tyrosine; UNIPERTAN P2002 and UNIPERTAN P27 (both available from Unichem); and mixtures thereof.

Brief Summary Text (134):
Skin lightening actives can actually decrease the amount of melanin in the skin or provide an such an effect by other mechanisms. Skin lightening actives suitable for use herein are described in copending patent application Ser. No. 08/479,935, filed on Jun. 7, 1995 in the name of Hillebrand, corresponding to PCT Application No. U.S. Ser. No. 95/07432, filed Jun. 12, 1995; and copending patent application Ser. No. 08/390,152, filed on Feb. 24, 1995 in the names of Kalla L. Kvalnes, Mitchell A. DeLong, Barton J. Bradbury, Curtis B. Motley, and John D. Carter, corresponding to PCT Application No. U.S. Ser. No. 95/02809, filed Mar. 1, 1995, published Sep. 8, 1995; all incorporated herein by reference. Nonlimiting examples of skin lightening actives useful herein include adapalene, aloe extract, alpha-glycaryl-L-ascorbic acid, aminotyroxine, ammonium lactate, anethole derivatives, apple extract, arbutin, areca catechu L. extract, ascorbic acid, ascorbyl palmitate, azelaic acid, bamboo extract, bearberry extract, bletilla tuber, bupleurum falcatum extract, burnet extract, Burnet Power (available from Barnet Products), butyl hydroxy anisole, butyl hydroxy toluene, butyl resoreinol, Chuanxiong, cola decaballo extract, Dang-Gui, deoxyarbutin, 1,3 diphenyl propane derivatives, 2,5 dihydroxybenzoic acid and its derivatives, 2-(4-acetoxyphenyl)-1,3 dithane, 2-(4-hydroxyphenyl)-1,3 dithane, ellagic acid, escinol, estragole derivatives, esculoside, esculetin, FADEOUT (available from Pentapharm), Fangfeng, fennel extract, gallic acid and its derivatives, ganodenna extract, gaoben, GATULINE WHITENING (available from Gattlefosse), genistic acid and its derivatives, gentisyl alcohol, glabridin and its derivatives, gluco pyranosyl-1-ascorbate, gluconic acid, glucosamine, glycolic acid, glycyrrhizinic acid, green tea extract, 4-Hydroxy-5-methyl-3[2H]-furanone, hydroquinine, 4 hydroxyanisole and its derivatives, 4-hydroxy benzoic acid derivatives, hydroxycaprylic acid, hyptis extract, inositol ascorbate, kojic acid, kojic dipalnitate, lactic acid, lemon extract, licorice extract, Licorice P-TH (available from Barnet Products), linoleic acid, magnesium ascorbyl phosphate, Melfade (available from Pentapharm), MELAWHITE (available from Pentapharm), Melanostatine DM (available from Laboratories Seporga), morus alba extract, mulberry root extract, niacinamide, 5-octanoyl salicylic acid, parsley extract, phellinus linteus extract, pinon blanco extract, pinon negro extract, piri-piri extract, pyrogallol derivatives, retinoic acid, retinol, retinyl esters (acetate, propionate, palmitate, linoleate), 2,4 resorcinol derivatives, 3,5 resorcinol derivatives, rose fruit extract, rucinol, salicylic acid, Song-Yi extract, Sophora Powder (available from Barnet Products), 4-thioresorein, 3,4,5 trihydroxybenzyl derivatives, tranexamic acid, tyrostat (Rumex Extract available from Fytokem), Tyroslat 10,11 (available from Fytokem), vanilla derivatives, vitamin D.sub.3 and its analogs, and mixtures thereof.

Brief Summary Text (141):
Sebum inhibitors can decrease the production of sebum by the sebaceous glands. Nonlimiting examples of sebum inhibiting actives include aluminium hydroxy chloride, ASEBIOL (available from Laboratories Serobiologiques), BIODERMINE (available from Sederma), climbazole, COMPLETECH MBAC-OS (available from Lipo), corticosteroids, cucumber extracts, dehydroacetic acid and its salts, dichlorophenyl imidazoldioxolan (available from Elubiol), gugulipiu, ketoconazole, Lichochalcone LR 15 (available from Maruzen), niacinamide, phloretin, PHLOROGINE (available from Secma), Phycosaccharide Anti-Acne (available from Codif), S-carboxylmethyl cysteine, sepicontrol AS, spironolactone, tioxolone, tocopherol, tranexamic acid, UNITRIENOL T27 (available from Unichem), zincidone (UC1B), and mixtures thereof.

Brief Summary Text (142):
Protease Inhibitors:

Brief Summary Text (143):
Also useful as active ingredients in the present invention are protease inhibitors.

Nonlimiting examples of protease inhibitors which are useful in the compositions of the present invention are those selected from the group consisting of A E Complex (available from Barnet Products); ALE (available from Seporga); allicin; alpha lupaline; Aosaine (available from Secma); Aprotinin (available from Pentapharm); areca catechu (Betel Nut) extract; areca catechu extracts; Blue Algae Extract (available from Collaborative Labs); Centaurium (available from Sederma); cholesterol sulfate; CMST (available from Bioetica); Dermoprotectine (available from Sederma); Disacoside HF 60 (available from Barnet Products); Elhibin (available from Pentapharm); Fluid Out Colloid (available from Vegetech); Hypotaurine (available from Sogo Pharmaceutical); In Cyte Heathes (available from Collaborative Labs); Micromerol (available from Collaborative Labs); Pefabloc SP (available from Pentapharm); Sepicontrol AS (available from Seppic); Siegesbeckia (available from Sederma); Sophorine (available from Barnet Products); Thiotaine (available from Barnet Products); uncaria gambis roxburgh extract; zinc and mixtures thereof.

Brief Summary Text (151):
Nonlimiting examples of hair growth inhibitors which are useful in the compositions of the present invention include 17-beta estradiol, adamantyguanidines, adamantylamidines, adenylosuccinate synthase inhibitors, anti angiogenic steroids, aspartate transcarbamylase inhibitors, betamethasone valerate, bisabolol, copper ions, curcuma extract, cycloxygenase inhibitors, cysteme pathway inhibitors, dehydroacetic acid, dehydroepiandrosterone, diopyros leak extract, epidermal growth factor, epigallocatechin, essential fatty acids, evening primrose oil, gamma glutamyl transpeptidase inhibitors, ginger oil, glucose metabolism inhibitors, glutamine metabolism inhibitors, glutathione, green tea extracts, heparin, Kapilanne (available from International Sourcing Distributor), L, 5 diaminopentanoic acid, L-aspargine synthase inhibitors, linoleic acid, lipoxygenase inhibitors, longa extract, mimosinamine dihydrochloride, mimosine, nitric oxide synthase inhibitors, non-steroidal antiinflamatories, ornithine decarboxylase inhibitors, omthine aminotransferase inhibitors, panthenol, phorhetur, phosphodiesterase inhibitors, pleione extract, protein kinase C inhibitors, salpha reductase inhibitors, sulfhydral reactive compounds, tioxolone, transforming growth factor beta 1, urea, zinc ions and mixtures thereof.

Brief Summary Text (155):
Nonlimiting examples of desquamating enzyme enhancers which are useful in the compositions of the present invention include alanine, aspastic acid, N methyl serine, serine, trimethyl glycine and mixtures thereof.

Brief Summary Text (166):
A wide variety of emulsifiers are useful herein and include, but not limited to, those selected from the group consisting of sorbitan esters, glyceryl esters, polyglyceryl esters, methyl glucose esters, sucrose esters, ethoxylated fatty alcohols, hydrogenated castor oil ethoxylates, sorbitan ester ethoxylates, polymeric emulsifiers, and silicone emulsifiers.

Brief Summary Text (168):
Other suitable emulsifiers for use in the present invention include, but is not limited to, glyceryl monoesters, preferably glyceryl monoesters of C16-C22 saturated, unsaturated and branched chain fatty acids such as glyceryl oleate, glyceryl monostearate, glyceryl monopalmitate, glyceryl monobehenate, and mixtures thereof; polyglyceryl esters of C16-C22 saturated, unsaturated and branched chain fatty acids, such as polyglyceryl-4 isostearate, polyglyceryl-3 oleate, diglycerol monooleate, tetraglycerol monooleate and mixtures thereof; methyl glucose esters, preferably methyl glucose esters of C16-C22 saturated, unsaturated and branched chain fatty acids such as methyl glucose dioleate, methyl glucose sesquiisostearate, and mixtures thereof; sucrose fatty acid esters, preferably sucrose esters of C12-C22 saturated, unsaturated and branched chain fatty acids such as sucrose stearate, sucrose trilaurate, sucrose distearate (e.g., CRODESTA.RTM. F10), and mixtures thereof; C12-C22 ethoxylated fatty alcohols such as oleth-2, oleth-3, steareth-2, and mixtures thereof; hydrogenated castor oil ethoxylates such as PEG-7 hydrogenated castor oil; sorbitan ester ethoxylates such as PEG-40 sorbitan peroleate, Polysorbate-80, and mixtures thereof; polymeric emulsifiers such as ethoxylated dodecyl glycol copolymer; and silicone emulsifiers such as laurylmethicone copolyol, cetyldimethicone, dimethicone copolyol, and mixtures

thereof

Brief Summary Text (183):
Cellulose derived polymers are also useful herein as deposition aids. By cellulose derived polymers, as used herein, is meant to describe those polymers containing a cellulose backbone, i.e. a polysaccharide backbone of repeating glucose units. In these cellulose derived polymers, the hydroxy groups of the cellulose polymer have been hydroxyalkylated (preferably hydroxyethylated or hydroxypropylated) to form a hydroxyalkylated cellulose which is then further modified with a cationic quaternary ammonium or protonated ammonium group. Preferred cationic modifying groups are those having at least one C.sub.10-20 alkyl chain and two shorter alkyl chains (i.e. C.sub.1 or C.sub.2) on the nitrogen. The substituent on the cellulose polymer can thus be depicted as --(X)NRR'R" wherein X is hydroxyalkyl (preferably--OCH.sub.2 CH.sub.2 -- or --OCH.sub.2 CHOHCH.sub.2 --), R and R' are methyl or ethyl, and R" is C.sub.10-20 alkyl [preferably lauryl, stearyl, or cocoyl (i.e. a mixture of alkyl groups derived from coconut oil)]. In other alternative structures it has been found that when R, R', and R" are all methyl (i.e. the trimonium group) that useful cellulose polymers are also obtained. In yet other alternative structures the cationic substituent on the cellulose contains both a hydroxyethyl and a hydroxypropyl group such that the moiety can be depicted as --(OCH.sub.2 CH.sub.2 O)--CH.sub.2 CHOHCH.sub.2 NRR'R" wherein R, R', and R" are methyl or ethyl, and R" is C.sub.10-20 alkyl [preferably lauryl, stearyl, or cocoyl (i.e. a mixture of alkyl groups derived from coconut oil)], or alternatively wherein R, R', and R" are all methyl (i.e. the trimonium group).

Brief Summary Text (186):
Related to these cellulose polymers are ones having backbones that are derived from other sugars (or their related acids, alcohols, amines, etc.), e.g. galactose, mannose, arabinose, xylose, fucose, fructose, glucosamine, galactosamine, glucuronic acid, galacturonic acid, 5 or 6 membered ring polyalcohols, and mixtures thereof.

Brief Summary Text (187):
Protein derived Polymers are another type of useful derivative of a naturally occurring polymer. The protein derived polymers useful herein are derived from a wide variety of protein sources. However, those that are derived from hydrolyzed proteins (i.e. proteins which are broken down into lower molecular weight segments of from about 1000 MW to about 5000 MW) are preferred. Hydrolyzed proteins are well known to the cosmetic chemist of ordinary skill in the art and can be derived using standard synthetic techniques such as the acid, alkaline, or enzymatic hydrolysis of various protein sources. The protein source used will determine the ultimate amino acid composition of the hydrolyzed protein obtained. Nonlimiting examples of hydrolyzed proteins which are useful as polymers herein include those selected from the group consisting of hydrolyzed casein, hydrolyzed collagen, hydrolyzed conchiorin protein, hydrolyzed corn protein, hydrolyzed elastin, hydrolyzed fibronectin, hydrolyzed hair keratin, hydrolyzed human placental protein, hydrozlyed keratin, hydrolyzed potato protein, hydrolyzed rice protein, hydrolyzed silk, hydrolyzed soy protein, hydrolyzed vegetable protein, hydrolyzed wool protein, hydrolyzed wheat protein, and mixtures thereof. These hydrolyzed proteins are described in the CTFA International Cosmetic Ingredient Dictionary, 1991, pp. 246-249, which are incorporated by reference herein in their entirety.

Brief Summary Text (188):
It has been found that cationically modified hydrolyzed proteins are especially useful polymers in the present invention. Using a variety of synthetic techniques known to the artisan of ordinary skill in the chemical arts, the nitrogen atoms of the amino acids comprising these hydrolyzed proteins can be hydroxyalkylated (preferably hydroxyethylated or hydroxypropylated) to form a hydroxyalkylated protein hydrolyzate which is then further modified with a cationic quaternary ammonium or protonated ammonium group. Preferred cationic modifying groups are those having at least one C.sub.10-20 alkyl chain and two shorter alkyl chains (i.e. C.sub.1 or C.sub.2) on the nitrogen. The substituent on the hydrolyzed protein can be depicted as --(X)NRR'R" wherein X is hydroxyalkyl (preferably--OCH.sub.2 CH.sub.2 -- or --OCH.sub.2 CHOHCH.sub.2 --), R and R' are methyl or ethyl, and R" is C.sub.10-20 alkyl [(preferably lauryl, stearyl, or cocoyl (i.e. a mixture of alkyl groups derived from coconut fats)]. In other alternative structures it has been

found that when R, R', and R" are all methyl (i.e. the trimonium group) that useful cationic hydrolyzed proteins are also obtained. Commercially available cationic modified protein hydrolyzates include: hydroxypropyltrimonium hydrolyzed casein, hydroxypropyltrimoniurn hydrozlyed collagen, hydoxypropyltrimonium hydrolyzed keratin, hydroxypropyltrimonium hydrolyzed silk, hydroxypropyl trimonium hydrolyzed soy protein, hydroxypropyl trimonium hydrolyzed vegetable protein, and hydroxypropyltrimonium hydrolyzed wheat protein, wherein the --(X)NRR'R" substituent on each of these protein hydrolyzates is such that X is --OCH.sub.2 CHOHCH.sub.2 --, and R, R', and R" are methyl. These hydrolyzed proteins are described in the CTFA International Cosmetic Ingredient Dictionary, 1991, pp. 254-255, which are incorporated by reference herein in their entirety. Other commercially available cationic modified protein hydrolyzates include lauryldimonium hydroxypropyl hydrolyzed collagen, lauryldimonium hydroxypropyl hydrolyzed keratin, lauryldimonium hydroxypropyl hydrolyzed silk, lauryldimonium hydroxypropyl hydrolyzed soy protein, stearyldimonium hydroxypropyl hydrolyzed casein, stearyldimonium hydroxypropyl hydrolyzed collagen, stearyldimonium hydroxypropyl hydrolyzed keratin, stearyldimonium hydroxypropyl hydrolyzed rice protein, stearyldimonium hydorxypropyl hydrolyzed silk, stearyldimonium hydroxypropyl hydrolyzed vegetable protein, stearyldimonium hydroxypropyl hydrolyzed wheat protein, cocodimonium hydroxypropyl hydrolyzed casein, cocodimonium hydroxypropyl hydrolyzed collagen, cocodimonium hydroxypropyl hydrolyzed keratin, cocodimonium hydroxypropyl hydrolyzed rice protein, cocodimonium hydroxypropyl hydrolyzed silk, cocodimonium hydroxypropyl hydrolyzed soy protein, cocodimnoium hydroxypropyl hydrolyzed wheat protein, wherein in each of these protein hydrolyzates the --(X)NRR'R" substituent is such that X is --OCH2CHOHCH2--, R and R' are methyl, and R" is lauryl or stearyl or cocoyl. These hydrolyzed proteins are described in the CTFA International Cosmetic Ingredient Dictionary, 1991, pp. 112-113, 293-294, 586, which are incorporated by reference herein in their entirety. Preferred among these cationic hydrolyzed proteins are lauryldimmonium hydroxypropyl hydrolyzed collagen, lauryldimmonium hydroxypropyl hydrolyzed keratin, lauryldimmonium hydroxypropyl hydrolyzed keratin, lauryldimmonium hydroxypropyl hydrolyzed silk, lauryldimmonium hydroxypropyl hydrolyzed soy protein, and mixtures thereof.

Brief Summary Text (215):
Nonlimiting examples of conditioning agents useful as oil soluble conditioning agents include those selected from the group consisting of mineral oil, petrolatum, C7-C40 branched chain hydrocarbons, C1-C30 alcohol esters of C1-C30 carboxylic acids, C1-C30 alcohol esters of C2-C30 dicarboxylic acids, monoglycerides of C1-C30 carboxylic acids, diglycerides of C1-C30 carboxylic acids, triglycerides of C1-C30 carboxylic acids, ethylene glycol monoesters of C1-C30 carboxylic acids, ethylene glycol diesters of C1-C30 carboxylic acids, propylene glycol monoesters of C1-C30 carboxylic acids, propylene glycol diesters of C1-C30 carboxylic acids, C1-C30 carboxylic acid monoesters and polyesters of sugars, polydialkylsiloxanes, polydiarylsiloxanes, polyalkarylsiloxanes, cylcomethicones having 3 to 9 silicon atoms, vegetable oils, hydrogenated vegetable oils, polypropylene glycol C4-C20 alkyl ethers, di C8-C30 alkyl ethers, and mixtures thereof.

Brief Summary Text (221):
Also useful are various C1-C30 monoesters and polyesters of sugars and related materials. These esters are derived from a sugar or polyol moiety and one or more carboxylic acid moieties. Depending on the constituent acid and sugar, these esters can be in either liquid or solid form at room temperature. Examples of liquid esters include: glucose tetraoleate, the glucose tetraesters of soybean oil fatty acids (unsaturated), the mannose tetraesters of mixed soybean oil fatty acids, the galactose tetraesters of oleic acid, the arabinose tetraesters of linoleic acid, xylose tetralinoleate, galactose pentaoleate, sorbitol tetraoleate, the sorbitol hexaesters of unsaturated soybean oil fatty acids, xylitol pentaoleate, sucrose tetraoleate, sucrose pentaoletate, sucrose hexaoleate, sucrose hepatoleate, sucrose octaoleate, and mixtures thereof. Examples of solid esters include: sorbitol hexaester in which the carboxylic acid ester moieties are palmitoleate and arachidate in a 1:2 molar ratio; the octaester of raffinose in which the carboxylic acid ester moieties are linoleate and behenate in a 1:3 molar ratio; the heptaester of maltose wherein the esterifying carboxylic acid moieties are sunflower seed oil fatty acids and lignocerate in a 3:4 molar ratio; the octaester of sucrose wherein the esterifying carboxylic acid moieties are oleate and behenate in a 2:6 molar

ratio; and the octaester of sucrose wherein the esterifying carboxylic acid moieties are laurate, linoleate and behenate in a 1:3:4 molar ratio. A preferred solid material is sucrose polyester in which the degree of esterification is 7-8, and in which the fatty acid moieties are C18 mono- and/or di-unsaturated and behenic, in a molar ratio of unsaturates:behenic of 1:7 to 3:5. A particularly preferred solid sugar polyester is the octaester of sucrose in which there are about 7 behenic fatty acid moieties and about 1 oleic acid moiety in the molecule. Other materials include cottonseed oil or soybean oil fatty acid esters of sucrose. The ester materials are further descnbed in, U.S. Pat. No. 2,831,854, U.S. Pat. No. 4,005,196, to Jandacek, issued Jan. 25, 1977; U.S. Pat. No. 4,005,195, to Jandacek, issued Jan. 25, 1977, U.S. Pat. No. 5,306,516, to Lefton et al., issued Apr. 26, 1994; U.S. Pat. No. 5,306,515, to Letton et al., issued Apr. 26, 1994; U.S. Pat. No. 5,305,514, to Letton et al., issued Apr. 26, 1994; U.S. Pat. No. 4,797,300, to Jandacek et al., issued Jan. 10, 1989; U.S. Pat. No. 3,963,699, to Rizzi et al, issued Jun. 15, 1976; U.S. Pat. No. 4,518,772, to Volpenhein, issued May 21, 1985; and U.S. Pat. No. 4,517,360, to Volpenhein, issued May 21, 1985; all of which are incorporated by reference herein in their entirety.

Brief Summary Text (226):
Nonlimiting examples of conditioning agents useful as water soluble conditioning agents include those selected from the group consisting of polyhydric alcohols, polypropylene glycols, polyethylene glycols, ureas, pyrolidone carboxylic acids, ethoxylated and/or propoxylated C3-C6 diols and triols, alpha-hydroxy C2-C6 carboxylic acids, ethoxylated and/or propoxylated sugars, polyacrylic acid copolymers, sugars having up to about 12 carbons atoms, sugar alcohols having up to about 12 carbon atoms, and mixtures thereof. Specific examples of useful water soluble conditioning agents include materials such as urea; guanidine; glycolic acid and glycolate salts (e.g. ammonium and quaternary alkyl ammonium); lactic acid and lactate salts (e.g. ammonium and quaternary alkyl ammonium); sucrose, fructose, glucose, eruthrose, erythritol, sorbitol, mannitol, glycerol, hexanetriol, propylene glycol, butylene glycol, hexylene glycol, and the like; polyethylene glycols such as PEG-2, PEG-3, PEG-4, PEG-5, PEG-6, PEG-8, PEG-9, PEG-10, PEG-15 PEG-30, PEG-50, polypropylene glycols such as PPG-9, PPG-12, PPG-15, PPG-17, PPG-20, PPG-26, PPG-30, PPG-34; alkoxylated glucose; hyaluronic acid; and mixtures thereof. Also useful are materials such as aloe vera in any of its variety of forms (e.g., aloe vera gel), chitin, starch-grafted sodium polyacrylates such as SANWET (RTM) IM-1000, IM-1500, and IM-2500 (available from Celanese Superabsorbent Materials, Portsmouth, Va.); lactamide monoethanolamine; acetamide monoethanolamine; and mixtures thereof. Also useful are propoxylated glycerols as described in propoxylated glycerols described in U.S. Pat. No. 4,976,953, to Orr et al., issued Dec. 11, 1990, which is incorporated by reference herein in its entirety. Other useful water soluble conditioning agents include arginine, arginine asparate (available from Ajinomoto), ARGININE PCA (available from Argidone-UCIB), 1,3 butylene glycol, CHITOLAM NB/101 (available from Lamberti), chitosan salts, Codiavelane (available from Secma), COLLAGEN AMINO ACID (available from Crotein CAA-Croda), creatine, dextran, dextrin, diglycerol, dipropylane glycol, ectoines, erythritol, FUCOGEL (available from Solabia), fructose, glucamine salts, glucose glutamate (commercially available as WICKENOL 545 from Caschem), glucuronic acid salts, glutamic acid salts, glycereth 12, glycereth 20, glycereth 7, glycerin, glyceryl PCA, glycogen, hexylene glycol, honey, hyaluronic acid, hydrogenated honey, hydrogenated starch hydrolysates, hydrolyzed mucopolysaccharides, hydroxy proline, Indinyl CA (available from Laboratoires Serobiologiques), inositol, keratin amino acids (commercially available as CROTEIN HKP from Croda), konjac mannan, Larex A-200 (available from Larex), LYSINE PLA (commercially available as LYSIDONE from UCIB), maltitol, maltose, mannitol, mannose, Mariscan (available from Pentapharm), Melhydrin (available from Laborotories Serobiologiques), methoxy PEG 10, methoxy, methyl gluceth 10 (commercially available as GLUCAM E10 from Amerchol), methyl gluceth 20 (commercially available as GLUCAM E20 from Amerchol), methyl glucose, 3 methyl 1,3 butandiol, N acetyl glucosamine salts, panthenol, PEG 15 butanediol, butanediol, PEG 5 pentaerythitol, pentaerythitol, Pentaglycan (available from Pentapharm), 1,2 pentanediol, phytohyaluron (jute extract), polyglycerol sorbitol, PPG 1 glyceryl ether, proline, propylene glycol, 2 pyrrolidone-5-carboxylic acid and its salts, saccharide isomerate (commercially available as PENTAVITIN from Pentapharm), Seacare (available from Secma), Sericin (available from Pentapharm), serine, silk amino acids (commercially available as CROSLIK LIQUID from Croda), sodium

·acetylhyaluronate, sodium hyaluronate, sodium polyaspartate (commercially available as AQUADEW SPA-30 from Ajinomoto), sodium polyglutamate (commercially available as AJICOAT SPG from Ajinomoto), sorbeth 20, sorbeth 6, sorbitol, trehalose, triglycerol, trimethyolpropane, tris (hydroxymethyl) amino methane salts, xylitol, xylose, and mixtures thereof.

Brief Summary Text (235):
Any material may be used to increase the hardness value of the skin care active component provided that the following criteria are met: (i) the material must be soluble in the skin care actives of the skin care active component and (ii) the material must have a melting point of greater than 20.degree. C. (e.g., be a solid at room temperature). Examples of suitable hardening materials include, but are not limited to, petrolatum, highly branched hydrocarbons, fatty alcohols, fatty acid esters, vegetable oils, hydrogenated vegetable oils, polypropylene glycols, alpha-hydroxy fatty acids, fatty acids having from about 10 to about 40 carbon atoms, alkyl amides of di and/or tri-basic carboxylic acids, n-acyl amino acid derivatives, and mixtures thereof. Hardening materials useful in the present invention are further described in U.S. Pat. No. 4,919,934, to Deckner et al., issued Apr. 24 1990, which is incorporated herein by reference in its entirety.

Brief Summary Text (241):
Suitable alkyl amides of di and/or tri-basic carboxylic acids for use herein include disubstituted or branched monoamides, monosubstituted or branched diamides, triamides, and mixtures thereof. Some specific examples of alkyl amides of di- and tri-basic carboxylic acids include, but are not limited to, alkyl amides of citric acid, tricarballylic acid, aconitic acid, nitrilotriacetic acid and itaconic acid such as 1,2,3-propane tributylamide, 2-hydroxy-1,2,3-propane tributylamide, 1-propene-1,2,3-trioctylamide, N,N',N"-tri(methyldecylamide)amine, 2 docecyl-N,N'-dibutylsuccinamide, and mixtures thereof. Other suitable amides include the n-acyl amino acid derivatives described in U.S. Pat. No. 5,429,816, issued to Hofrichter et al. on Jul. 4, 1995.

Brief Summary Text (246):
Additional non-limiting examples of useful hardening materials are those selected from the group consisting of sorbitan esters, glyceryl esters, polyglyceryl esters, methyl glucose esters, sucrose esters, ethoxylated fatty alcohols, hydrogenated castor oil ethoxylates, sorbitan ester ethoxylates, polymeric emulsifiers, and silicone emulsifiers.

Brief Summary Text (248):
Other suitable hardeners for use in the present invention include, but is not limited to, glyceryl monoesters, preferably glyceryl monoesters of C16-C22 saturated, unsaturated and branched chain fatty acids such as glyceryl oleate, glyceryl monostearate, glyceryl monopalmitate, glyceryl monobehenate, and mixtures thereof; polyglyceryl esters of C16-C22 saturated, unsaturated and branched chain fatty acids, such as polyglyceryl-4 isostearate, polyglyceryl-3 oleate, diglycerol monooleate, tetraglycerol monooleate and mixtures thereof; methyl glucose esters, preferably methyl glucose esters of C16-C22 saturated, unsaturated and branched chain fatty acids such as methyl glucose dioleate, methyl glucose sesquiisostearate, and mixtures thereof; sucrose fatty acid esters, preferably sucrose esters of C12-C22 saturated, unsaturated and branched chain fatty acids such as sucrose stearate, sucrose trilaurate, sucrose distearate (e.g., CRODESTA .RTM. F10), and mixtures thereof; C12-C22 ethoxylated fatty alcohols such as oleth-2, oleth-3, steareth-2, a mixtures thereof; hydrogenated castor oil ethoxylates such as PEG-7 hydrogenated castor oil; sorbitan ester ethoxylates such as PEG-40 sorbitan peroleate, polysorbate-80, and mixtures thereof; polymeric emulsifiers such as ethoxylated dodecyl glycol copolymer; and silicone emulsifiers such as laurylmethicone copolyol, cetyldimethicone, dimethicone copolyol, and mixtures thereof.

Brief Summary Text (262):
Nonlimiting examples of suitable surfactants include silicone based surfactants, e.g., polyalkeleneoxide modified polydimethyl siloxane; fluoroaliphatic surfactants, e.g., perfluoroalkyl polyalkylene oxides; and other surfactants, e.g., actyl-phenoxypolyethoxy ethanol nonionic surfactants, alkylaryl polyether alcohols,

and polyethylene oxides. Commercially available surfactants suitable for the present invention include various poly(ethylene oxide) based surfactants available under the tradename TRITON, e.g., grade X-102, from Rohm and Haas Corp.; various polyethylene glycol based surfactants available under the tradename EMEREST, e.g., grades 2620 and 2650, from Emery Indust.; various polyalkylene oxide modified polydimethylsiloxane based surfactants available under the tradename SILWET, e.g., grade Y12488, from OSI Specialty Chemicals; and alkenyl succinamide surfactants available under the tradename LUBRIZOL, e.g., grade OS85870, from Lubrizol Corp.; and polyoxyalkylene modified fluoroaliphatic surfactants available from Minnesota Mining and Manufacturing Co. The amount of surfactants required and the hydrophilicity of the modified substrate or fibers of the substrate for each application will vary depending on the type of surfactant selected and the component polymers used. In general, the surfactant may be added, topically or internally, in the range of from about 0.1 to about 5%, preferably from about 0.3% to about 4%, by weight of the substrate or the fibers of the substrate.

Brief Summary Text (272):
Nonlimiting examples of useful thickening agents of the present invention are selected from the group consisting of fatty alcohols, fatty acids, fatty alcohol ethoxylates having an average degree of ethoxylation ranging from 2 to about 30, sorbitan esters, glyceryl esters, polyglyceryl esters, methyl glucose esters, sucrose esters, sorbitan ester ethoxylates, natural and synthetic waxes, polyacrylic and hydrophobically modified polyacrylic resins, starches, gums, cellulose ethers, polycationic polymers, nonionic polymers, polyethylene glycols (PEG), and mixtures thereof.

Brief Summary Text (273):
Nonlimiting examples of useful thickening agents in the present invention include stearic acid, behenic acid, stearyl alcohol, cetyl alcohol, sorbitan monooleate, sorbitan sesquioleate, sorbitan monoisostearate, sorbitan stearates, sorbitan triooleate, sorbitan tristearate, sorbitan dipalmitates, sorbitan isostearate, glyceryl oleate, glyceryl monostearate, glyceryl monopalmitate, glyceryl monobehenate, polyglyceryl-4 isostearate, polyglyceryl-3 oleate, diglycerol monooleate, tetraglycerol monooleate, methyl glucose dioleate, methyl glucose sesquiisostearate, sucrose stearate, sucrose trilaurate, sucrose distearate oleth-2, oleth-3, steareth-2, PEG-40 sorbitan peroleate, Polysorbate-80, beeswax, polyethylene wax, CARBOPOL, PEMULEN, corn starch, potato starch, tapioca, guar gum, gum arabic, hydroxypropyl cellulose, hydroxyethyl cellulose, carboxymethyl cellulose, RETEN 201, KYMENE 557H.RTM., ACCO 7112, CARBOWAX.

CLAIMS:

1. A disposable, single use personal care cleansing article comprising:

(A) a water insoluble substrate,

(B) from about 0.5% to about 40%, by weight of said insoluble substrate, of at least one lathering surfactant added onto or impregnated into said substrate, and

(C) from about 0.001% to about 50%, by weight of said insoluble substrate, of a skin care active component comprising at least one skin care active selected from the group consisting of anti-acne actives; anti-wrinkle, anti-skin atrophy and skin repair actives; skin barrier repair actives; non-steroidal cosmetic soothing actives; artificial tanning agents and accelerators; skin lightening actives; sunscreen actives; sebum stimulators; sebum inhibitors; anti-oxidants; protease inhibitors; skin tightening agents; anti-itch ingredients; hair growth inhibitors; 5-alpha reductase inhibitors; desquamating enzyme enhancers; anti-glycation agents; and mixtures thereof, which skin care active component is added onto or impregnated into said substrate,

wherein said article is substantially dry, and wherein said article is capable of generating an Average Lather Volume of greater than or equal to about 30 ml.

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DOCUMENT-IDENTIFIER: US 6248712 B1
TITLE: Urokinase-type plasminogen activator receptor

Brief Summary Text (9):
Many research groups have proposed that invasive tumor cells secrete matrix-degrading proteinases. A cascade of proteases including serine proteases and thiol proteases all contribute to facilitating tumor invasion. One of the crucial cascades is the plasminogen activation system. Regulation of the proteolysis can take place at many levels including tumor cell-host cell interactions and protease inhibitors produced by the host or by the tumor cells themselves. Expression of matrix-degrading enzymes is not tumor cell specific. The actively invading tumor cells may merely respond to different regulatory signals compared to their non-invasive counterparts (Liotta, 1986).

Brief Summary Text (15):
The cellular receptor for u-PA (u-PAR) was originally identified in blood monocytes and in the monocyte-like U937 cell line (Vassalli et al., 1985), and its presence has been demonstrated on a variety of cultured cells, including several types of malignant cells (Stoppelli et al., 1985, Vassalli et al., 1985, Plow et al., 1986, Boyd et al., 1988a, Nielsen et al., 1988), human fibroblasts (Bajpai and Baker, 1985), and also in human breast carcinoma tissue (Needham et al., 1987). The receptor binds active 54 kD u-PA, its one-polypeptide chain proenzyme, pro-u-PA (see below), as well as 54 kD u-PA inhibited by the active site reagent DFP, but shows no binding of the low molecular weight (33 kD) form of active u-PA (Vassalli et al., 1985; Cubellis et al., 1986). Thus, binding to the receptor does not require the catalytic site of u-PA, and in agreement with these findings, the binding determinant of u-PA has been identified in the amino-terminal part of the enzyme, in a region which in the primary structure is remote from the catalytic site. The receptor binding domain is located in the 15 kD amino-terminal fragment (ATF, residues 1-135) of the u-PA molecule, more precisely within the cysteine-rich region termed the growth factor region as this region shows homologies to the part of epidermal growth factor (EGF) which is responsible for binding to the EGF receptor. The amino acid residues which appear to be critical for binding are located within the sequence 12-32 (numbered 1-21 in SEQUENCE ID NO:32) of u-PA. (Appella et al., 1987). Synthetic peptides have been constructed that inhibit the binding of very low (100 nM) concentrations. The lack of cross-reactivity between the murine and the human peptides indicates that the binding between u-PA and u-PAR is strongly species specific.

Brief Summary Text (16):
Binding of u-PA to u-PAR is specific in the sense that as yet no other protein has been found to compete for binding to the receptor, though several proteins structurally related to u-PA, including t-PA and plasminogen, have been tested (Stoppelli et al., 1985, Vassalli et al., 1985, Nielsen et al., 1988). Fragments of u-PA containing only the receptor binding domain, e.g. ATF, ensure specificity of the binding to the receptor, since other molecules that might bind u-PA (protease nexin and the specific plasminogen activator inhibitors PAI-1 and PAI-2) recognize the catalytically active region (Stoppelli et al., 1985; Nielsen et al., 1988). PAI-1 is able to form a covalent complex with u-PA but not with pro-u-PA (Andreasen et al., 1986).

Brief Summary Text (24):

In the intact organism, pro-u-PA is the predominant form of u-PA in intracellular stores, and it also constitutes a sizable fraction of the u-PA in extracellular fluids (Skriver et al., 1984, Kielberg et al., 1985). Extracellular activation of pro-u-PA may therefore be a crucial step in the physiological regulation of the u-PA pathway of plasminogen activation. The plasmin-catalyzed activation of pro-u-PA provides a positive feedback mechanism that accelerates and amplifies the effect of activation of a small amount of pro-u-PA. The initiation of the u-PA pathway of plasminogen activation under physiological conditions, however, involves triggering factors that activate pro-u-PA as described herein. Mutants of human single-chain pro-u-PA in which lysine 158 is changed to another amino acid (e.g. Glu or Gly) are not, or are only to a small extent, converted to active two-chain u-PA (Nelles et al., 1987).

Brief Summary Text (28):
Plasminogen, as well as plasmin, binds to many types of cultured cells, including thrombocytes, endothelial cells and several cell types of neoplastic origin (Miles and Plow, 1985, Hajjar et al., 1986, Plow et al., 1986, Miles and Plow 1987, Burtin and Fondaneche, 1988). The binding is saturable with a rather low affinity for plasminogen (K.sub.D 1 .mu.M). At least in some cell types, binding of plasmin appears to utilize the same site as plasminogen, but the binding parameters for plasmin indicate that more than one type of binding site for plasminogen and plasmin may exist. Thus, on some cell types, plasmin and plasminogen bind with almost equal affinity (Plow et al., 1986), while on others plasmin apparently binds with a higher affinity (K.sub.D 50 nM) than plasminogen (Burtin and Fondaneche, 1988). The binding is inhibited by low amounts of lysine and lysine analogues and appears to involve the kringle structure of the heavy chains of plasminogen and plasmin (Miles et al., 1988).

Brief Summary Text (40):
The new findings include the requirement, in the presence of serum, for binding of plasminogen, the ability of bound u-PA under these conditions to activate plasminogen, the presence of pro-u-PA on the cells, the ability of bound plasmin to activate pro-u-PA, and the ability of endogenous plasminogen activator inhibitor PAI-1, as well as added plasminogen activator inhibitor PAI-2, to regulate the surface plasminogen activation. By these means tumor cells can acquire the broad-spectrum proteolytic activity of plasmin, bound to their surface in such a way that it is protected from inactivation by serum protease inhibitors, and ideally situated to be employed in the degradation of the pericellular matrix.

Brief Summary Text (42):
Human tumor cells are very commonly found to secrete plasminogen activator of the urokinase type (u-PA). By this means they are able to recruit the proteolytic potential available in the high concentration of plasminogen in plasma and other body fluids. The invasive properties of tumor cells may be at least partly dependent on their proteolytic capability mediated through the broad spectrum of activity of plasmin and including its indirect actions in activating other latent proteases, such as collagenases. The expression of protease activity by tumor cells facilitates their penetration of basement membranes, capillary walls and interstitial connective tissues, allowing spread to other sites and establishment of metastases.

Brief Summary Text (48):
The purified protein could be chemically cross-linked with u-PA. Its amino acid composition and N-terminal sequence were determined (30 residues, some of which with some uncertainty). It was found to be heavily N-glycosylated, deglycosylation resulting in a protein with an apparent molecular weight of about 30-35 kD. The apparent molecular weight of u-PAR from different cell lines and from PMA-stimulated and non-stimulated U937 cells varied somewhat. This heterogeneity disappeared after deglycosylation and was thus due to differences in glycosylation of u-PAR from the various sources.

Brief Summary Text (52):
The deduced amino acid sequence indicated that u-PAR is produced as a 313 residues long protein with a 282 residues long hydrophilic N terminal part (probably extracellular) followed by 21 rather hydrophobic amino acids (probably a trans-membrane domain). The potential extracellular part is organised in 3 repeats

with striking homologies, particularly with respect to the pattern of cysteines. This may indicate the presence of distinct domains that may bind different ligands.

Brief Summary Text (56):
In Example 7 it is demonstrated that after incubation of monolayer cultures of human HT-1080 fibrosarcoma cells with purified native human plasminogen in serum containing medium, bound plasmin activity can be eluted from the cells with tranexamic acid, an analogue of lysine. The bound plasmin is the result of plasminogen activation on the cell surface; plasmin activity is not taken up onto cells after deliberate addition of plasmin to the serum containing medium. The cell surface plasmin formation is inhibited by an anti-catalytic monoclonal antibody to u-PA, indicating that this enzyme is responsible for the activation.

Brief Summary Text (58):
The binding and subsequent protection of plasmin was abolished by low concentrations of the lysine analogue, tranexamic acid. It is therefore likely that plasmin binding involves the lysine affinity sites situated in the heavy-chain kringles of plasmin. Plasmin released from the cells was partially inactivated in the serum medium. As long as the plasmin remained bound, it was protected from serum inhibitors but could be inhibited by aprotinin or an anti-catalytic monoclonal antibody.

Brief Summary Text (78):
The enzyme urokinase-type plasminogen activator (u-PA) has only one well-defined macromolecular substrate, namely plasminogen. By cleavage at Arg.sup.560, plasminogen is activated to the broad spectrum protease plasmin. By the term "preventing u-PA from converting plasminogen into plasmin" is therefore meant that this activation by u-PA is substantially inhibited or a situation where the activation is sufficiently inhibited so as to inhibit or reduce the undesired effect of the plasmin.

Brief Summary Text (120):
The present invention also relates to pure u-PAR. As mentioned above, pure u-PAR has been made for the first time in accordance with the present invention. Pure u-PAR in glycosylated form shows, in an SDS-PAGE at a load of approximately 1 .mu.g, substantially one and only one silver stained band having an apparent molecular weight in the range of about 55-60 kD. The presence of substantially one and only one silver stained band in this SDS-PAGE is a proof of the purity of the u-PAR. Another proof of the purity of the u-PAR is the presence of a single amino-terminal amino acid sequence in purified u-PAR preparations. While it has been found that different cells may produce u-PARs having different glycosylation, the glycosylated u-PARs, upon deglycosylation, were all found to have an identical electrophoretic mobility (corresponding to substantially one and only one band at about 30-35 kD in an SDS-PAGE), indicating that the peptide part of the molecule is identical in all cases.

Brief Summary Text (121):
As appears from the Examples, pure u-PAR in glycosylated form may be prepared from a biological material containing u-PAR by temperature-induced phase separation of detergent extracts followed by affinity chromatography purification with immobilized DFP-u-PA. The detergent is preferably a non-ionic detergent such as a polyethylene glycol ether, e.g. Triton X-114. The temperature was found to be relatively critical in the range of 34-40.degree. C., such as about 37.degree. C., for 10 minutes.

Brief Summary Text (124):
On the basis of the amino-terminal amino acid sequence of pure u-PAR, a 24-mer nucleotide probe was synthesized and used to screen a library to identify and isolate recombinant clones carrying the cDNA for u-PAR. The identity of the cDNA clones was confirmed by comparing the nucleotide sequence of this cDNA clone with the amino terminal sequence of the purified u-PAR, and by expressing said cDNA in mouse L cells and assaying their u-PA-binding properties.

Brief Summary Text (125):
The abbreviations of the amino acids used herein are the following:

Brief Summary Text (126):

One aspect of the invention relates to a polypeptide comprising a characteristic amino acid sequence derived from a u-PAR which polypeptide comprises at least 5 amino acids and up to the complete sequence of u-PAR as shown below as the DNA sequence (SEQ ID NO:22) and the deducted amino acid sequence (SEQ ID NO:23) of the clone p-uPAR-1. The signal peptide is underlined and the first 30 amino acids, the sequence of which has been determined on the purified protein with an Applied Biosystems gas phase sequencer (see Example 1), are overlined. The putative transmembrane domain is doubly underlined. The star symbols indicate the potential N-linked glycosylation sites.

Brief Summary Text (128):
The invention relates to any polypeptide comprising at least 5 amino acids and up to the complete sequence of u-PAR from amino acid 1 to 313, and any analogue to such a polypeptide.

Brief Summary Text (130):
In the present context, the term "characteristic amino acid sequence derived from the u-PAR" is intended to mean an amino acid sequence, such as an epitope, which comprises amino acids constituting a substantially consecutive stretch (in terms of linear or spatial conformation) in u-PAR, or amino acids found in a more or less non-consecutive conformation in u-PAR, which amino acids constitute a secondary or tertiary conformation having interesting and useful properties, e.g. as therapeutics or diagnostics. Thus, amino acids present at different positions in u-PAR but held together e.g. by chemical or physical bonds, e.g. by disulphide bridges, and thereby forming interesting tertiary configurations are to be understood as "characteristic amino acid sequences". The characteristic amino acid sequence may comprise a consecutive subsequence of the amino acid sequence of u-PAR of greater or smaller length or a combination of two or more parts of such subsequences which may be separated by one or more amino acid sequences not related to u-PAR. Alternatively, the characteristic amino acid sequences may be directly bonded to each other.

Brief Summary Text (132):
The term "analogue" is used in the present context to indicate a protein or polypeptide of a similar amino acid composition or sequence as the characteristic amino acid sequence derived from the u-PAR, allowing for minor variations which do not have an adverse effect on the immunogenicity of the analogue. The analogous polypeptide or protein may be derived from mammals or may be partially or completely of synthetic origin.

Brief Summary Text (133):
The present invention also relates to a substantially pure polypeptide which is recognized by an antibody raised against or reactive with a polypeptide comprising the amino acid sequence defined above.

Brief Summary Text (134):
In the present context, the term "substantially pure" is understood to mean that the polypeptide in question is substantially free from other components, e.g. other polypeptides or carbohydrates, which may result from the production and/or recovery of the polypeptide or otherwise be found together with the polypeptide. The high purity of the polypeptide of the invention is advantageous when the polypeptide is to be used for, e.g., the production of antibodies. Also due to its high purity, the substantially pure polypeptide may be used in a lower amount than a polypeptide of a conventional lower purity for most purposes. The purification of the polypeptide of the invention may be performed by methods known to a person skilled in the art, but particularly the low concentrations of u-PAR in biological material and the strongly hydrophobic nature of the receptor has hitherto hampered its purification. Now, however, the combination of temperature-induced phase separation of detergent extracts of cells and affinity chromatography with immobilized DFP-treated u-PA has led to its successful purification in amounts high enough (100-200 .mu.g) to have enabled a partial amino acid sequencing and further characterization.

Brief Summary Text (138):
The polypeptide of the invention may also be a fusion protein in which characteristic amino acid sequence(s) from u-PAR is/are fused to another polypeptide sequence. The polypeptide to which the characteristic amino acid sequence(s) from

u-PAR is/are fused may be one which results in an increased expression of the protein when expressed in an organism, or facilitates or improves the purification and recovery of the fusion protein from said organism in terms of a more easy and economical recovery, or confers to the u-PAR the property of inhibiting u-PA (as it would be in the case of a u-PAR-PAI-1 fusion).

Brief Summary Text (139):
In some cases, it may be advantageous to cleave the fusion protein so as to obtain a polypeptide which substantially solely comprises characteristic amino acid sequence(s) from u-PAR. In these cases, the characteristic amino acid sequence(s) from u-PAR is/are preferably fused to a polypeptide sequence which may be specifically recognized by a cleaving agent, e.g. a chemical such as cyanogen bromide, hydroxylamine and 2-nitro-5-thiocyanobenzoate, or an enzyme, e.g. a peptidase, proteinase or protease, e.g. trypsin, chlostripain, and staphylococcal protease or factor Xa.

Brief Summary Text (149):
The DNA fragment of the invention may comprise a nucleotide sequence encoding a polypeptide fused in frame to the nucleotide sequence encoding the characteristic amino acid sequence with the purpose of producing a fused polypeptide. When using recombinant DNA technology, the fused sequence may be inserted into an appropriate vector which is transformed into a suitable host organism. Alternatively, the DNA fragment of the invention may be inserted in the vector in frame with a gene carried by the vector, which gene encodes a suitable polypeptide. The host organism, which might be of eukaryotic or prokaryotic origin, for instance a yeast or a mammalian cell line, is grown under conditions ensuring expression of the fused sequence after which the fused polypeptide may be recovered from the culture by physico-chemical procedures, and the fused polypeptide may be subjected to gel filtration and affinity chromatography using an antibody directed against the antigenic part(s) of the fused polypeptide. After purification, the polypeptide of the invention and the polypeptide to which it is fused may be separated, for instance by suitable proteolytic cleavage, and the polypeptide of the invention may be recovered, e.g. by affinity purification or another suitable method.

Brief Summary Text (151):
The DNA fragment described above may be obtained directly from genomic DNA or by isolating mRNA and transferring it into the corresponding DNA sequence by using reverse transcriptase producing cDNA. When obtaining the DNA fragment from genomic DNA, it is derived directly by screening for genomic sequences, hybridizing to a DNA probe prepared on the basis of the full or partial amino acid sequence of u-PAR. When the DNA is of complementary DNA (cDNA) origin, it may be obtained by preparing a cDNA library on the basis of mRNA from cells containing a u-PAR or parts thereof. Hybridization experiments may then be carried out using synthetic oligonucleotides as probes to identify the cDNA sequence encoding the u-PAR or part thereof. cDNA differs from genomic DNA in, e.g. that it lacks certain transcriptional control elements and introns which are non-coding sequences within the coding DNA sequence. These elements and introns are normally contained in the genomic DNA. The DNA fragment may also be of synthetic origin, i.e. prepared by conventional DNA synthesizing method, e.g. by using a nucleotide synthesizer. The DNA fragment may also be produced using a combination of these methods.

Brief Summary Text (158):
The present invention also relates to a method of producing the polypeptides described above. Suitably, the polypeptides are prepared using recombinant DNA-technology e.g. the methods disclosed in Maniatis et al. op. cit. More specifically, the polypeptides may be produced by a method which comprises cultivating or breeding an organism carrying a DNA-fragment encoding a characteristic amino acid sequence from an u-PAR, e.g. the above described DNA fragment, under conditions leading to expression of said DNA fragment, and subsequently recovering the polypeptide from the organism.

Brief Summary Text (159):
As described above, the organism which is used for the production of the polypeptide may be a higher organism, e.g. an animal, or a lower organism, e.g. a microorganism. Irrespective of the type of organism employed for the production of the polypeptide,

the DNA fragment encoding the characteristic amino acid sequence from an u-PAR should be introduced in the organism. Conveniently, the DNA fragment is inserted in an expression vector, e.g. a vector as defined above, which is subsequently introduced into the host organism. The DNA fragment may also be directly inserted in the genome of the host organism. The insertion of the DNA fragment in the genome may be accomplished by use of a DNA fragment as such or cloned in bacteria, phage lambda or other vectors, carrying the DNA fragment and being capable of mediating the insertion into the host organism genome. The insertion of the DNA fragment into an expression vector or into the genome of the host organism may be accomplished as described e.g. by Colbere-Garapin F. et al., J. Molec. Biol., 150; 1-14 (1981): A New Dominant Hybrid Selective Marker for Higher Eucaryotic Cells.

Brief Summary Text (162):
When the polypeptide of the invention comprises one or more distinct parts, e.g. being a fusion protein comprising on the one hand characteristic amino acid sequence(s) from u-PAR and on the other hand amino acid sequence(s) constituting a polypeptide which is not related to u-PAR, the DNA fragments encoding each of these polypeptides may be inserted in the genome or expression vector separately or may be coupled before insertion into the genome or expression vector by use of conventional DNA techniques such as described in Maniatis et al. op. cit.

Brief Summary Text (170):
c) Preparative electrophoresis procedures; for instance the following procedure: A supernatant from a centrifuged enzyme treated cell or cell line preparation is subjected to a gel electrophoresis, such as a sodium dodecyl sulphate-polyacrylamidgel electrophoresis (SDS-PAGE) (cf. Laemmli, U.K. Nature, 227:680-685; 1970), or an agarose gel electrophoresis. Subsequently, labelled antibodies, such as monoclonal antibodies, reactive with u-PAR, are used to identify bands primarily constituted by the isolated u-PAR compounds. For instance, the antibodies may be used in any conventional immunoblotting technique. The markers may be isotopes or fluorescein labels detectable by means of relevant sensitive films. After identification, the u-PAR containing bands of the gel may be subjected to a treatment resulting in the release of the u-PAR compounds from the gels, such as procedures involving slicing up the gel and subsequent elution of u-PAR compounds. Optionally, the amino acid sequence of the u-PAR proteins obtained may be determined.

Brief Summary Text (172):
Prior to cultivation of the microorganism, the DNA fragment encoding the polypeptide of the invention may be subjected to modification, before or after the DNA fragment has been inserted in the vector. The polypeptide produced may also be subjected to modification. The modification may comprise substitution, addition, insertion, deletion or rearrangement of one or more nucleotides and amino acids in the DNA fragment and the polypeptide, respectively, or a combination of these modifications. The term "substitution" is intended to mean the replacement of any one or more amino acids or nucleotides in the full amino acid or nucleotide sequence with one or more others, "addition" is understood to mean the addition of one or more amino acids or nucleotides at either end of the full amino acid or nucleotide sequence, "insertion" is intended to mean the introduction of one or more amino acids or nucleotides within the full amino acid or nucleotide sequence, and "deletion" is intended to indicate that one or more amino acids or nucleotides have been deleted from the full amino acid or nucleotide sequence whether at either end of the sequence or at any suitable point within it. "Rearrangement" is intended to indicate that one or more amino acids or nucleotides or the sequence has been exchanged with each other. The DNA fragment may, however, also be modified by subjecting the organism carrying the DNA fragment to mutagenization, preferably site directed mutagenization so as to mutagenize said fragment. When the organism is a microorganism, the mutagenization may be performed by using conventional mutagenization means such as ultraviolet radiation, ionizing radiation or a chemical mutagen such as mitomycin C, 5-bromouracil, methylmethane sulphonate, nitrogen mustard or a nitrofuran or mutagens known in the art, e.g. mutagens of the type disclosed in Miller, J. H., Molecular genetics, Unit III, Cold Spring Harbor Laboratory 1972.

Brief Summary Text (173):
Examples of suitable modifications of the DNA sequence are nucleotide substitutions

which do not give rise to another amino acid sequence of the protein, but which, e.g., correspond to the codon usage of the specific organism in which the sequence is inserted; nucleotide substitutions which give rise to a different amino acid sequence and therefore, possibly, a different protein structure without, however, impairing the critical properties of the polypeptide encoded by the DNA sequence; a subsequence of the DNA sequence shown above encoding a polypeptide which has retained the receptor properties of the native u-PAR; or a DNA sequence hybridizing to at least part of a DNA prepared on the basis of the DNA sequence shown above, provided that it encodes a polypeptide which has the biological property of u-PAR.

Brief Summary Text (175):
It is well-known that use of recombinant DNA-techniques, including transgenic techniques, may be associated with another kind of processing of the polypeptide than the processing of the polypeptide when produced in its natural environment. Thus, when a bacterium such as E. coli is used for the production of the polypeptide of the invention, the amino acid residues of the polypeptide are not glycosylated, whereas the polypeptide may be glycosylated when produced in another microorganism or organism.

Brief Summary Text (177):
The term "truncated polypeptide" refers to a polypeptide deleted for one or more amino acid residues eventually resulting in changing of the properties of the polypeptide, e.g. solubility. In a further meaning, the term "truncated polypeptide" refers to a mixture of polypeptides all derived from one polypeptide or expressed from the gene encoding said polypeptide. Such truncated polypeptides might arise for instance in vector/host cell systems in which part of the cDNA has been deleted by restriction enzyme digestion or other suitable methods, resulting in the expression of a protein not normally produced in that system.

Brief Summary Text (178):
Also, the polypeptide of the invention may be prepared by the well-known methods of liquid or solid phase peptide synthesis utilizing the successive coupling of the individual amino acids of the polypeptide sequence or the coupling of individual amino acids forming fragments of the polypeptide sequence which fragments subsequently are coupled so as to result in the desired polypeptide. The solid phase peptide synthesis may e.g. be performed as described by R. B. Merrifield, J. Am. Chem. Soc. 85, 1963, p. 2149. In solid phase synthesis, the amino acid sequence is constructed by coupling an initial amino acid to a solid support and then sequentially adding the other amino acids in the sequence by peptide bonding until the desired length has been obtained. In this embodiment, the solid support may also serve as the carrier for the polypeptide of the invention in a vaccine preparation as described below. The preparation of synthetic peptides may be carried out essentially as described in Shinnick, Ann. Rev. Microbiol. 37, 1983, pp. 425-446.

Brief Summary Text (191):
The diagnostic agent, may, e.g, be an antibody as defined above. Alternatively, the diagnostic agent may be in the form of a test kit comprising in a container a polypeptide comprising a characteristic amino acid sequence of u-PAR, e.g. a sequence including or included in the sequence (1). The diagnostic agent may be used in the diagnosis of diseases related to abnormal numbers of u-PARs residing on the cell.

Brief Summary Text (193):
Examples of enzymes useful as labels are .beta.-galactosidase, urease, glucose oxidase, carbonic anhydrase, peroxidases (e.g. horseradish peroxidase), phosphatases (e.g. alkaline or acid phosphatase), glucose-6-phosphate dehydrogenase and ribonuclease.

Brief Summary Text (194):
Enzymes are not in themselves detectable, but must be combined with a substrate to catalyze a reaction the end product of which is detectable. Thus, a substrate may be added to the reaction mixture resulting in a coloured, fluorescent or chemiluminescent product or in a colour change or in a change in the intensity of the colour, fluorescence or chemiluminescence. Examples of substrates which are useful in the present method as substrates for the enzymes mentioned above are

H.sub.2 O.sub.2, p-nitrophenylphosphate, lactose, urea, .beta.-D-glucose, CO.sub.2, RNA, starch, or malate. The substrate may be combined with, e.g. a chromophore which is either a donor or acceptor.

Brief Summary Text (198):
In an embodiment of the invention an antibody or a polypeptide of the invention may be coupled to a bridging compound coupled to a solid support. The bridging compound, which is designed to link the solid support and the antibody may be hydrazide, Protein A, glutaraldehyde, carbodiimide, or lysine.

Brief Summary Paragraph Table (1):
Three-letter One-letter Amino acid abbreviation symbol Alanine Ala A Arginine Arg R Asparagine Asn N Aspartic acid Asp D Asparagine or aspartic acid Asx B Cysteine Cys C Glutamine Gln Q Glutamic acid Glu E Glutamine or glutamic acid Glx Z Glycine Gly G Histidine His H Isoleucine Ile I Leucine Leu L Lysine Lys K Methionine Met M Phenylalanine Phe F Proline Pro P Serine Ser S Threonine Thr T Tryptophan Trp W Tyrosine Tyr Y Valine Val V

Drawing Description Text (3):
FIG. 1A) The Triton X-114 fraction containing membrane proteins from PMA-treated U937a cells was subjected to affinity chromatography using immobilized DFP-treated u-PA. The neutralized column eluate was dialyzed aginst 0.1% acetic acid and concentrated by lyophilization. A portion, representing 2.times.10.sup.8 cells before purification, was run on 6-16% gradient SDS-PAGE under reducing conditions (lane 1). The gel was silver-stained. The molecular weights of marker proteins (lane 2) are indicated.

Drawing Description Text (11):
FIGS. 7A-C. FIG. 7A shows the initial amino-terminal amino acid sequence (SEQ ID NO: 24) information and the oligonucleotide synthesized and used for library screening (SEQ ID NOS: 25 and 26); I stands for inosine. FIG. 7B shows the restriction map of p-uPAR-1 clone and the strategy employed for the complete double stranded sequence. FIG. 7C shows the hydrophobicity plot. The abscissa shows the amino acid residue position, the ordinate the degree of hydrophobicity calculated using the algorithm of Hopp and Wood (1981) and Kyte and Doolittle (1982).

Drawing Description Text (16):
FIG. 11 shows SDS-PAGE (12.5%) electrophoretic analysis of the p-u-PAR-PFLM-1 mutant transfected into LB6 cells. Cells were incubated with iodinated ATF, washed, extracted with Triton X-114, and an amount of extract corresponding to 300,000 cells cross-linked with DSS as described before and run on the gel (part C of the Figure). Similarly, conditioned medium was centrifuged at 100,000.times.g, and the supernatant (a volume corresponding to 15,000 cells) was incubated with iodinated ATF, cross-linked with DSS, and analyzed by SDS-PAGE (part B of the Figure). Lanes a and b are duplicates from cells grown at different densities.

Drawing Description Text (17):
FIGS. 12A-12B show an elution profile from cation-exchange chromatography of amino acids released from u-PAR after acid hydrolysis. The protein was initially purified from PMA-stimulated U937 cells (6.times.10.sup.9 cells) by Triton X-114 detergent-phase separation and affinity chromatography (DFP-u-PA Sepharose). To improve purity and eliminate interference on amino acid analysis from low molecular weight compounds, this receptor preparation was dialysed thoroughly against 0.1% acetic acid, lyophilized and then subjected to Tricine-SDS-PAGE followed by electrotransfer onto a 0.45 .mu.m PVDF-membrane (8 cm.times.8 cm). The insert shows the immobilized u-PAR after staining with Coomassie Brilliant Blue R-250. A slight decrease in mobility of u-PAR was observed in this experiment, due to a large excess of the zwitterionic detergent CHAPS in the lyophilized preparation. The stained area of the PVDF-membrane representing u-PAR was excised and hydrolysed in vacuo for 20 hours at 110.degree. in the presence of 3,3'-dithiodipropionic acid (DTDPA). Cys-X is the product formed between cysteine and DTDPA during hydrolysis, GlcN is glucosamine and EtN is ethanolamine.

Drawing Description Text (20):
FIGS. 15A-15C show the change in hydrophobic properties of purified u-PAR upon

treatment with PI-PLC. u-PAR, purified from PMA-stimulated U937 cells, were either untreated (NONE) FIG. 15A or incubated for 30 min at 37.degree. C. in 50 mM triethylamine/HCl (pH 7.5), 5 mM EDTA and 0.1% Triton X-100 without any phospholipases (MOCK) FIG. 15B or in the presence of 20 .mu.l/ml PI-PLC (PI-PLC) FIG. 15B. One sample was incubated with 200 .mu.g/ml phospholipase D purified from cabbage in 50 mM acetate (pH 6.0), 10 mM CaCl.sub.2 (PLD), and another with 100 .mu.g/ml phospholipase A.sub.2 purified from bee venom in 50 mM HEPES (pH 8.0), 10 mM CaCl.sub.2 (PLA.sub.2) FIG. 15C.

Drawing Description Text (21):
These u-PAR preparations were then subjected to temperature-induced detergent-phase separation in 1% Triton X-114. This phase separation was repeated once for the resulting aqueous and detergent phases by addition of extra Triton X-114 and 0.1 M Tris (pH 8.1), respectively. Finally, cross-linking analysis with 1 nM .sup.125 I-labelled ATF was performed on parallel aliquots of aqueous (A) and detergent (D) phases, followed by SDS-PAGE (10% T and 2.5% C) under non-reducing conditions. Areas corresponding to .sup.125 I-ATF/u-PAR complexes (Mr 70,000) were excised from the polyacrylamide gel and the radioactivity was determined (shown as % of total radioactivity in A+D at the bottom of each lane).

Drawing Description Text (22):
FIG. 16 shows a comparison of COOH-terminal amino acid sequences from proteins, in which the processing sites during GPI-membrane anchoring are known, to that predicted for u-PAR (SEQ ID NO: 21) (based on amino acid analysis, Table 5). The amino acids involved in attachment to the glycolipid are highlighted. VSG (SEQ ID NO: 17) and PARP (SEQ ID NO: 16) refers to variant surface glycoprotein (and procyclic acidic repetitive protein from Trypanosoma brucei. CEA (SEQ ID NO: 19) is carcinoembryonic antigen; PLAP (SEQ ID NO: 18) is placental alkaline phosphatase and Thy-1 (SEQ ID NO: 20) refers to the surface glycoprotein isolated from rat thymocytes.

Drawing Description Text (24):
FIG. 18 shows SDS-PAGE of detergent phase from Triton X-114 phase-separated extracts from U937 cells treated with PMA for different time periods, chemical cross linked to .sup.125 I-ATF. Non-treated cells and PMA (150 nM) treated cells were acid treated and lysed. The detergent phases were incubated with .sup.125 I-ATF, cross linked with DSS and run in a 6-16% SDS-PAGE gradient gel followed by autoradiography. Electrophoretic mobility of molecular weight standard proteins are indicated to the left. 1. Non-treated cells, 2. +PMA 3 hours, 3. +PMA 9 hours, 4. +PMA 24 hours, 5. +PMA 48 hours, 6. Blind, 7. 1% Triton X-114 total lysate (diluted 1/25) from HEp2 cells.

Drawing Description Text (26):
FIG. 20 shows SDS-PAGE of detergent phase from Triton X-114 phase-separated extracts from U937 cells treated with Dibuturyl cAMP for different time periods, chemical cross-linked to .sup.125 I-ATF. Non-treated cells and Dibuturyl cAMP (1 mM) treated cells were acid treated and lysed as described in Materials and Methods. The detergent phases were incubated with .sup.125 I-ATF, cross linked with DSS and run in a 6-16% SDS-PAGE gradient gel followed by autoradiography. Electrophoretic mobility of molecular weight standard proteins are indicated to the left. 1. Non-treated cells, 2. +Dibuturyl cAMP 12 hours, 3. +Dibuturyl cAMP 24 hours, 4. +Dibuturyl cAMP 48 hours, 5. +Dibuturyl cAMP 72 hours.

Drawing Description Text (28):
FIG. 22. Dependence of plasmin formation in serum medium on the concentration of added native human plasminogen. Confluent layers of HT-1080 cells were incubated for 3 hours in MEM medium (0.5 ml) containing 10% heat-inactivated and plasminogen-depleted fetal calf serum, with the addition of native human plasminogen to the concentrations shown. The conditioned media were harvested and the cells rinsed three times with PBS. The cells were treated with 1 mM tranexamic acid in PBS to obtain the bound fraction of plasmin. Plasmin was assayed in the cell-bound fraction (.smallcircle.--.smallcircle.) and the medium (.circle-solid.--.circle-solid.) as thioesterase activity.

Drawing Description Text (30):

FIG. 24. Plasmin release from HT-1080 cells into serum-free and serum-containing media. Confluent layers of HT-1080 cells were first loaded with plasmin by incubation for 1 hour at 37.degree. C. in serum-free MEM medium (0.5 ml) containing human plasmin (0-5 .mu.g/ml). After rinsing the cell layers three times, they were incubated for 2 hours at 37.degree. C. with either serum-free medium (.smallcircle.--.smallcircle.), medium containing 10% heat-inactivated and plasminogen-depleted fetal calf serum (.circle-solid.--.circle-solid.), or the latter with tranexamic acid (100 .mu.M) (.box-solid.--.box-solid.). Plasmin was then assayed in the cell-bound fraction (A) and the media (B). At the time of transfer to new media, there was approximately 28 ng of plasmin bound to the cells from the pretreatment with 2.5 .mu.g of plasmin/well.

Drawing Description Text (31):
FIG. 25. Effect of pretreatment of HT-1080 cells with DFP-u-PA on bound u-PA activity and ability to produce bound plasmin in serum medium. Confluent cell layers of HT-1080 cells were preincubated for 18 hours at 37.degree. C. with the concentrations shown of DFP-u-PA in serum-containing medium (0.5 ml). After rinsing three times, the cells were incubated for 1 hour at 37.degree. C. with MEM medium containing 10% heat-inactivated and plasminogen-depleted fetal calf serum, with addition of native human plasminogen (40 .mu.g/ml). After incubation, half the replicate wells were rinsed and treated with acid-glycine to recover the total bound u-PA (.smallcircle.--.smallcircle.) which now included DFP-u-PA, pro-u-PA and active u-PA. The other wells were used to recover bound plasmin (.circle-solid.--.circle-solid.) by elution with tranexamic acid.

Drawing Description Text (32):
FIG. 26. Activation of cell-bound u-PA proenzyme in serum medium after addition of plasminogen. Confluent layers of HT-1080 cells were prelabelled for 5 hours at 37.degree. C. with .sup.35 S-methionine. After restoring complete medium with 10% heat-inactivated and plasminogen-depleted fetal calf serum, native human plasminogen (50 .mu.g/ml) was added and the incubation continued for another 3 hours. Aprotinin (200 KIU/ml) was added before harvest of medium, and the rinsed cells were treated with acid-glycine to recover the bound u-PA fraction. Acid eluates were neutralized and immunoprecipitated with goat antibodies to u-PA, before SDS-PAGE under reducing conditions. The fluorogram shows: in lane 1, control immunoprecipitate of culture without plasminogen with goat antibodies to human t-PA; lane 2, culture without plasminogen immunoprecipitated with goat anti-u-PA antibodies; lane 3, culture with plasminogen immunoprecipitated with u-PA antibodies.

Drawing Description Text (33):
FIG. 27. Activation of cell-bound u-PA proenzyme in serum medium after the addition of plasminogen. Confluent layers of HT-1080 cells were incubated with MEM medium containing 10% heat-inactivated and plasminogen-depleted fetal calf serum and native human plasminogen (40 .mu.g/ml). After the time intervals shown, aprotinin (200 KIU/ml) was added and the rinsed cells were treated with acid-glycine to recover the bound fraction of u-PA. The u-PA in the neutralized eluate was assayed by an immunocapture method, using an NPGB inactivation step to determined the pro-u-PA index (see Methods). FIG. 27A shows the pro-u-PA index for cultures without (.smallcircle.--.smallcircle.) and with (.circle-solid.--.circle-solid.) plasminogen. The zero-time sample with plasminogen shows that some change already occurred during work-up of the cells. FIG. 27B shows the eluted u-PA activity from cultures without plasminogen (.smallcircle.--.smallcircle.), with plasminogen (.circle-solid.--.circle-solid.), and with plasminogen and a neutralizing monoclonal antibody to human PAI-1 (10 .mu.g/ml) (.box-solid.--.box-solid.).

Drawing Description Text (34):
FIG. 28. Model for cell surface plasminogen activation. In this proposed model, u-PA receptors (u-PA-R) and plasminogen receptors (plg-R) are depicted on the cell membrane. Before exposure to plasminogen (plg), virtually all the bound u-PA is present as pro-u-PA (open squares), but it is assumed that some active u-PA molecules exist (closed squares). On plasminogen (open rectangles) binding (which may be precluded by the presence of tranexamic acid), plasmin (pl, closed rectangles) is formed on the cell by the action of the bound active urokinase. This step may be inhibited by PAI-1 and PAI-2, and by an anti-catalytic monoclonal antibody to u-PA (anti-u-PA-ab). The bound plasmin thus formed is resistant to

inhibition by the alpha-2-anti-plasmin present in the serum medium, but sensitive to inhibition by aprotinin and an anti-catalytic monoclonal antibody to plasmin (anti-pl-ab). As active plasmin becomes available, it catalyzes the activation of more bound pro-u-PA to active u-PA, thus amplifying the proteolytic system. Activation of pro-u-PA is inhibited by tranexamic acid (which prevents plasminogen binding), aprotinin and an anti-catalytic monoclonal antibody to plasmin.

Drawing Description Text (56):
FIG. 45B: Western blot showing the reactivity of the antisera used. 500 ng of purified u-PAR (lanes 2 and 4) or the Triton X-114 detergent phase obtained from 2.5.times.10.sup.6 PMA-stimulated U937 cells (lanes 1 and 3) were analyzed by SDS-PAGE under reducing conditions on a 6-16% gradient gel, and Western blotting using as the primary antisera mouse anti-u-PAR serum diluted 1:250 (lanes 1 and 2) or the above control serum at the same dilution (lanes 3 and 4).

Drawing Description Text (57):
FIGS. 46A-B show a Western blot, demonstrating the reactivity of polyclonal rabbit antibody against u-PAR. 75 .mu.l samples of Triton X-114 detergent phase from lysates of PMA-stimulated U937 cells were analyzed alone (lane 1), after mixing with DFP-treated u-PA (Example 1; final concentration 10 .mu.g/ml) (lane 4), or after mixing with the same amount of DFP-treated u-PA, followed by chemical cross-linking (lane 3). As a control, the same amount of DFP-treated u-PA was analyzed alone, after the performance of cross-linking (lane 5), or directly (lane 6). The sample in lane 2 contained 75 .mu.l of the cell lysate detergent phase, which was subjected to chemical cross-linking without the addition of DFP-treated u-PA. The samples were run on 6-16% gradient SDS-PAGE under non-reducing conditions, followed by electroblotting onto nitrocellulose. The sheets were incubated with purified and absorbed IgG from rabbit anti-u-PAR serum (FIG. 46A), or with purified and absorbed IgG from pre-immune serum from the same rabbit (FIG. 46B). The IgG concentration during the incubation was 12 .mu.g/ml in both cases. The sheets were developed with alkaline phosphatase-coupled antibody against rabbit IgG, followed by detection of alkaline phosphatase activity.

Detailed Description Text (6):
Tricine-SDS-PAGE of samples to be electroblotted for amino acid analysis or NH.sub.2 -terminal amino-acid sequencing was performed in a MINI-PROTEAN II.TM. amino acid sequencing apparatus (BioRad) according to Schagger and von Jagow, 1987, on a 0.75 mm homogeneous 7.7% T, 3% C gel. The gel was pre-electrophoresed for 3 hours at 15 mA in the gel buffer with 12 mM 3-mercaptopropanoic acid added as a scavenger. The freeze-dried sample was dissolved directly in 50 .mu.l of the sample buffer with 40 mM dithioerythritol as the reducing agent, and boiled for 2 minutes. The gel buffer used for pre-electrophoresis was replaced with electrophoresis buffer, after which electrophoresis was performed for 4 hours at 60 V.

Detailed Description Text (7):
Electroblotting of samples for amino acid analysis or NH.sub.2 -terminal amino acid sequencing. After electrophoresis, the Tricine-SDS-polyacrylamide gel was electroblotted onto a polyvinylidene difluoride (PVDF) membrane (Millipore), using a semi-dry electroblotting apparatus (JKA Instruments, Denmark). Electroblotting took place at pH 11.0 in 10 mM CAPS (3-(cyclohexylamino)-1-propanesulfonic acid), including 0.4 mM dithioerythritol and 10% methanol, and was performed at 0.8 mA/cm.sup.2 for 2 hours. The protein was localized by staining with Coomassie R250 for 2 minutes and brief destaining, followed by wash in water (Matsudaira, 1987).

Detailed Description Text (8):
Alkylation of electroblotted protein and amino acid sequencing. The Coomassie-stained protein band was cut out from the PVDF-membrane and treated with 25 mM iodoacetamide in 50 mM sodium borate, pH 8.0, for 1 hour in the dark at room temperature. After the reaction, it was washed extensively with water and dried under argon. The protein on the dried filter was sequenced on an Applied Biosystems protein sequencer, model 477A. The on-line HPLC identification system for the PTH amino acid derivatives included the derivative of carboxymethylcysteine (produced by deamidation of the amidomethyl derivative during conversion). The correct identification of this derivative was assured by a test-sequencing of chicken lysozyme (with cysteine at residue no. 6) after parallel preparative

electrophoresis, electroblotting and alkylation.

Detailed Description Text (9):
Determination of amino acid composition and amino sugars. For hydrolysis of electroblotted u-PAR, areas of PVDF membranes containing Coomassie-stained and in situ alkylated protein were treated with 6 M HCl containing 0.05% phenol for 20 h in vacuo at 110.degree. C. Amino acid analysis was performed on a Waters amino acid analyzer equipped with a post-column o-phthaldialdehyde identification system, as described (Barkholt and Jensen, 1989).

Detailed Description Text (14):
Cell lysis and detergent phase separation. PMA-stimulated U937a cells were washed and acid-treated as described by Nielsen et al., 1988. 20 ml lysis buffer (0.1 M Tris/HCl, pH 8.1, 1% Triton X114, 10 mM EDTA, 10 .mu.g/ml Aprotinin) and 0.2 ml 100 mM phenylmethylsulfonylfluoride in dimethylsulfoxide were added to 10.sup.9 acid-treated cells at 0.degree. C. The suspension was mixed thoroughly, left on ice for 5 minutes, mixed again and left at 0.degree. C. for another 5 minutes, after which it was clarified by centrifugation at 4.degree. C., 16,000.times.g for 10 minutes.

Detailed Description Text (17):
Lysates and detergent phases from other cell types (as indicated) were prepared in the same manner, except that smaller amounts of cell material were used. The amounts of all reagents were reduced proportionally. In one experiment, 0.5% CHAPS was used as the lysis detergent instead of 1% Triton X114. In that experiment, no phase separation was performed.

Detailed Description Text (18):
Preparation of affinity matrix. 2.5.times.10.sup.6 IU (approximately 25 mg) of u-PA (Serono) was dissolved in 25 ml 0.1 M Tris/HCl, pH 8.1, 0.1% Tween 80. The enzyme was inactivated by addition of 250 .mu.l of a fresh 500 mM stock solution of diisopropylfluorophosphate (DFP) in isopropanol and incubation for 4 hours at 37.degree. C., with a further addition of the same amount of DFP after the first 2 hours.

Detailed Description Text (19):
The reaction was stopped by extensive dialysis at 0.degree. C. against 0.25 M NaHCO.sub.3, 0.5 M NaCl, 0.1% Triton X-100, pH 8.5.

Detailed Description Text (21):
Affinity purification. The clarified detergent fraction obtained from 6.times.10.sup.9 U937a cells was diluted with 1 vol washing buffer-1 (10 mM sodium phosphate, 140 mM sodium chloride, 0.1% CHAPS, pH 7.4) and chromatographed on a column containing 8 ml DFP-u-PA-Sepharose, equilibrated with the same buffer. After application of the sample, the column was washed with washing buffer-1, followed by washing buffer-2 (10 mM sodium phosphate, 1 M sodium chloride, 0.1% CHAPS, pH 7.4). The column was eluted from below with elution buffer (0.1 M acetic acid, 0.5 M sodium chloride, 0.1% CHAPS, pH 2.5). Elution fractions were immediately titrated to pH 7.5 by addition of the appropriate volume of 0.1 M sodium phosphate, 1.0 M sodium carbonate, pH 9.0. u-PAR-containing fractions were identified by chemical cross-linking to the .sup.125 I-labelled amino terminal (ATF) fragment of urokinase, followed by SDS-PAGE and autoradiography. Purified u-PAR samples for amino acid analysis or NH.sub.2 -terminal amino acid sequencing were dialyzed against 0.1% acetic acid and lyophilized.

Detailed Description Text (22):
Protein labelling with .sup.125 I. .sup.125 I-labelling of ATF was performed as described previously (Nielsen et al., 1988), except that 0.1% Triton X100 was replaced by 0.01% Tween 80. Purified u-PAR, concentrated by freeze-drying after dialysis against 0.1% acetic acid, was iodinated in the same manner, except that 1.5 .mu.g protein was treated with 250 .mu.Ci .sup.125 I in a volume of 25 .mu.l.

Detailed Description Text (26):
For complete removal of N-bound carbohydrate, the samples were denatured under mildly reducing conditions by the addition of SDS and dithiothreitol to final

concentrations of 0.5% and 1.6 mM, respectively, and boiling for 3 minutes. Aliquots of the denatured samples (10 .mu.l) were adjusted to include 200 mM sodium phosphate, pH 8.6, 1.5% Triton X-100, 10 mM 1,10 phenanthroline (added from a methanol stock solution) and either 1 unit of peptide:N-glycosidase F (N-glycanase, Genzyme), or no enzyme, in a total volume 30 .mu.l. Deglycosylation was performed at 37.degree. C. for 20 hours. During studies on non-fractionated cell lysates obtained after lysis with CHAPS, 100 mm .beta.-mercaptoethanol was used for reduction instead of dithiothreitol, and 10 mM EDTA was included during deglycosylation instead of 1,10 phenanthroline.

Detailed Description Text (29):
Purification. PMA-stimulated U937a cells were acid-treated to remove any surface-bound u-PA and lysed in a Triton X114 containing buffer. The detergent extract was subjected to temperature-induced phase separation, and the isolated detergent phase was used as the raw material for affinity chromatography. The acid eluates were neutralized and analyzed, either directly or after concentration by dialysis against 0.1% acetic acid and lyophilization. The electrophoretic appearance of the purified material is shown in FIGS. 1A-C.

Detailed Description Text (33):
Quantification by amino acid analysis indicated a purification yield of 6-9 .mu.g polypeptide (corresponding to about 10-15 .mu.g u-PAR glycoprotein; see below) from 6.times.10.sup.9 cells.

Detailed Description Text (34):
Amino acid composition and NH.sub.2 -terminal amino acid sequences. The amino acid composition of the purified protein after preparative electrophoresis, electroblotting and alkylation with iodoacetamide is shown in table 1. This composition includes a strikingly high content of cysteine residues. Further, it is noted that rather few lysine residues are present. The analysis system employed allows the quantification of glucosamine and galactosamine in addition to the amino acids. Glucosamine was detected in an amount corresponding to approximately 30 mol of N-acetylglucosamine per mol protein, correcting for loss during hydrolysis. In contrast, no galactosamine was identified.

Detailed Description Text (35):
The high number of glucosamine residues detectable after acid hydrolysis, as well as the large decrease in apparent molecular mass following treatment with peptide:N-glycosidase F (see below), indicate that large side chains of N-linked carbohydrate are present in the protein. The failure to detect any galactosamine indicates that this type of O-linked carbohydrate is absent in u-PAR. However, the presence of other O-linked oligosaccharides that escape detection by amino acid analysis cannot be excluded.

Detailed Description Text (36):
Two amino acid sequencing experiments were performed. In the first sequencing experiment, direct NH.sub.2 -terminal sequencing of affinity-purified u-PAR was performed after dialysis and lyophilization. A partial sequence (Table 2A) was obtained, and it was demonstrated that only one sequence was present in the purified material.

Detailed Description Text (38):
As seen in Table 2, all amino acid residues identified proved identical when comparing the two sequences. Furthermore, positions 3, 6 and 12, which were identified only in the second experiment, all proved to be cysteines. Thus, the lack of any identification at these positions in the first experiment was to be ascribed to the lack of alkylation. It was clear that the only detectable NH.sub.2 -terminal sequence in the preparation was associated with the electrophoretic mobility of u-PAR. Consequently, no additional sequences were hidden in the form of, for example, low molecular weight peptide components associated with the major polypeptide chain.

Detailed Description Text (39):
A search in the Georgetown University protein data base did not reveal any identity, nor even pronounced homology, of the u-PAR NH.sub.2 -terminal amino acid sequence to

any known protein.

Detailed Description Text (40):
The amino terminus, like the amino acid composition of the entire protein, is rich in cysteine residues.

Detailed Description Text (41):
Data for probe construction (Example 2) were derived from the sequencing shown in Table 2A. For this construction, position 6 of the amino acid sequence was tentatively assigned Asn; see footnote a of Table 2A.

Detailed Description Text (59):
Samples to be analyzed by chemical cross-linking to .sup.125 I-ATF were 50-fold diluted in 0.1 M Tris/HCl, 1% Triton X-114, pH 8.1. The diluted samples were either clarified by addition of 0.25% w/v CHAPS (final concentration) or subjected to a single round of temperature induced phase separation (see Example 1). After the phase separation of 1 vol. of diluted sample, each phase (i.e., the detergent and buffer fraction, respectively) was made up to 1 vol. by addition of 0.1 M Tris/HCl, pH 8.1, and clarified by addition of 0.25% CHAPS (final concentration).

Detailed Description Text (61):
Enzymatic deglycosylation with N-Glycanase (Genzyme) was performed according to example 1, except that the actual concentrations during the deglycosylation step were the following: 0.08% SDS; 0.26 mM dithiothreitol; 0.11 M sodium phosphate; 0.9% Triton X-100; 5.3 mM 1,10 phenanthroline; 33.3 units/ml N-glycanase.

Detailed Description Text (63):
Direct confirmation of the identity of the 16 kD chymotryptic fragment of u-PAR (see "Results" below) to the binding domain of the receptor requires a cross-linking experiment using non-labelled DFP-u-PA or ATF as the ligand and analysis by SDS-PAGE and silver staining, using the metods already adopted (see Example 1). For further analyses, the fragment will be generated on a preparative scale (i.e., using purified protein in the range of 20-50 .mu.g as the starting material). The N-terminal amino acid sequence of the fragment will be obtained by the methods described in Example 1 (i.e., Tricine SDS-PAGE, electroblotting and amino acid sequencing). Identification of the fragment will subsequently be done by comparison to the amino acid sequence derived from u-PAR cDNA. For a closer identification of the binding determinant, synthetic peptides covering the chymotryptic fragment will be constructed. The peptides will be assayed for their potential inhibitory activity against the binding reaction between u-PAR and the ligand, as studied by cell binding assays (Nielsen et al., 1988; Appella et al., 1987) or by chemical cross-linking assay.

Detailed Description Text (67):
In parallel, the samples were analyzed in the chemical cross-linking assay, using .sup.125 I-ATF as the ligand (FIG. 5). While the non-degraded samples (lanes 4 and 5) showed the 70-75 kD conjugate band which is characteristic for the intact u-PAR (see Example 1), the intensity of this band was much reduced in the degraded samples (lanes 1-3). In contrast, the degraded samples showed an approx. -30 kD cross-linked conjugate; i.e. the size to be expected for a conjugate formed between the above mentioned, 16 kD u-PAR degradation product and the 15 kD ATF. The presence of a minor binding activity corresponding to intact u-PAR was ascribed to the cleavage being slightly incomplete; compare to the molecular weight pattern of FIG. 4. When analysis was preceeded by phase separation in the Triton X-114 system, it came out that th 30 kD conjugate was formed by a product preferentially present in the buffer phase, whereas the binding activity corresponding to intact u-PAR partitioned into the detergent phase (not shown).

Detailed Description Text (69):
In conclusion, the only detectable u-PAR fragment in the lower molecular weight (i.e., below 40 kD) region, formed by chymotrypsin in the concentration range tested, was a 16 kD product, consistent with the expected size for the fragment with binding activity observed after cross-linking to .sup.125 I-ATF. Unlike the intact u-PAR, the ligand binding fragment proved hydrophilic in the Triton X-114 system, suggesting that this fragment does not include the diacylglycerol part of the

protein (see Example 4). The deglycosylation experiment showed that the ligand binding fragment is glycosylated and suggested that the polypeptide part of the fragment comprised only 6-10 kD, corresponding to approx. 50-90 amino acid residues.

Detailed Description Text (76):
The library was screened with synthetic oligonucleotide probes made on the basis of amino acid sequence data from purified receptor protein (Tables 4-5). The melting temperatures were calculated from Lathe, J. Mol. Biol. 183: 1-12, 1985. The equation used was modified from:

Detailed Description Text (79):
The hybridization conditions were then further tested in pilot experiments to maximize the signal to noise ratio. Briefly, nitrocellulose filters containing DNA from the plasmid library were hybridized to the end-labelled oligonucleotide probe at various temperatures and salt concentrations (all within the range calculated from Lathe, supra). The filters were produced according to Grunstein and Hogness ("Colony hybridization: A method for the isolation of cloned DNAs that contain a specific gene", Proc. Natl. Acad. Sci. USA 72: 3961, 1975). The hybridization conditions to be used for the screening were chosen as the ones giving the minimum amount of background hybridization. In Table 3, the amino acid sequence derived frm a preliminary amino-terminal sequencing of purified u-PAR (see Example 1) and the derived oligonucleotide sequence are presented.

Detailed Description Text (81):
Initially, the plasmid library was screened with the N-terminal probe using the procedure of Crunstein and Hogness (supra). The detailed procedure is described below. Several positive clones were found but after the third rescreening, only one remained. The purity of the clone was checked and DNA was prepared from it (see large scale DNA preparation below). The DNA was digested with several different restrictions enzymes, and a map of the restriction sites found in the clone was constructed (see procedure in Maniatis et al., Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, 1982). The insert was further analysed by DNA sequencing (see procedure below). The clone was able to code for 7 out of the 8 amino acids in the N-terminal peptide used to construct the 24-mer probe. The sequence in the probe starts with an A whereas the clone had a T in this position, resulting in the substitution of Cys for Met. The clone was thus isolated by a specific hybridization but could not code for the correct peptide.

Detailed Description Text (99):
The complete sequence of one of the isolated clones (p-u-PAR-1) was obtained on double-stranded DNA in both orientations using commercial primers for pEMBL18 (M13 primers) and internal synthetic primers (see above). The sequence is shown in Sequence (1) in the Detailed Description of the invention. The restriction map and the sequencing strategy are illustrated in FIGS. 7A-C. The cDNA clone is 1364 nucleotides long from the 5' end to the beginning of the polyA stretch. At the 5' end, 46 nucleotides precede the first ATG codon which is followed by a 1005 nucleotides sequence with an open reading frame, ending with a nonanucleotide containing two in frame stop codons. 312 nucleotides of 3' untranslated sequence separate the first stop (TAA) codon from the polyA sequence. The assignment of the ATG at nucleotide 47 as the translation start site agrees with the consensus for initiating regions (Kozak, 1987) as discussed above. The translated sequence starts with a hydrophobic sequence which conforms to the rules for the signal peptide (von Heijne, 1986) (see above). The putative signal peptide is followed by 313 amino acid residues. The sequence shown in Sequence (1) was compared with the initial amino terminal sequence (FIG. 7A), and it was observed that in fact the original sequence contained an error at position 6 (Asn instead of Cys) which, however, did not prevent the isolation of the right cDNA clone. This is in fact proven by the 25/26 matches of the sequence derived from the cDNA with the definitive N-terminal protein sequence (see Example 1) determined in the course of this study after carboxymethylation and electroblotting of the purified protein [the region of homology is underlined in sequence (1)]. The calculated amino acid content agrees well with the one measured on the U937 protein (see Example 1). Also the calculated molecular weight (34,633) agrees well with the migration of the deglycosylated protein (see Example 1).

Detailed Description Text (100):
The human u-PAR is a relatively small protein of 313 amino acid residues. The amino acid sequence contains five potential N-linked glycosylation sites, in agreement with the high level of glycosylation of the protein (see Example 1). Starting at amino acid position 282, a sequence of 21 hydrophobic amino acids flanked by arginine residues may represent a membrane spanning domain of the u-PAR (FIG. 7C). At the C-terminal (possibly intracellular) side of the presumptive membrane-spanning segment, the arginine is followed by 9 additional hydrophobic amino acids ending with a carboxy-terminal threonine. Because of the high hydrophobicity of the ten carboxy-terminal residues, u-PAR may contain no intracytoplasmic domain at all, i.e. also the carboxy-terminal 10 residues may be buried in the membrane. The sequence of the carboxy-terminal about 30 amino acid residues would also be compatible with a signal peptide for glycolipid-anchored, phospholipase C-sensitive membrane attachment (Ferguson and Williams, 1988). The u-PAR is a slightly acidic protein (6 net acid charges), is very rich in cysteine, rich in glycine and leucine, and poor in lysine. The u-PAR is also rich in serine and threonine residues, which might indicate O-linked glycosylation (Russell et al., 1984). However, deglycosylation and sugar composition studies indicate that the receptor contains only N-linked carbohydrates (see Example 1).

Detailed Description Text (102):
Further studies of the u-PAR amino acid sequence revealed that the entire extracellular portion of the molecule is organized into three homologeous cysteine rich domains (1-92, 93-191, and 192-281) as follows:

Detailed Description Text (103):
(Amino acid residues that are identical in at least two of the repeats are indicated through underlining and italics while conservative substitutions are indicated with italics only).

Detailed Description Text (129):
Receptors are anchored at the plasma membrane by a stretch of hydrophobic amino acids (the trans-membrane domain) or through a glycolipid anchor. Most integral membrane proteins have a single trans-membrane domain, although cases have been described of multiple trans-membrane domains. In many cases, the trans-membrane domain is present in the middle of the protein sequence, i.e. between the carboxy terminal portion (generally intracellular) and the amino terminus (generally extracellular, containing the binding site for the ligand in the case of most receptors). A carboxy-terminal hydrophobic region is also a signal for glycolipid-anchor processing.

Detailed Description Text (130):
The available information on the structure of the u-PAR indicates that it is a protein of about 35,000 daltons, i.e. about 330 amino acids.

Detailed Description Text (131):
An amino acid sequence compatible with both a trans-membrane domain and a glycolipid anchor signal is present at the carboxy terminus.

Detailed Description Text (132):
In order to produce a soluble receptor, it is necessary to modify the protein in such a way as to eliminate the hydrophobic, membrane-spanning domain or the glycolipid anchor signal, while retaining both the signal sequence for secretion and the extracellular, ligand-binding portion of the u-PAR. To this end, two constructions have been made. In one of these, the carboxy-terminal 8 last amino acids have been eliminated by inserting a stop codon at the unique PFLM-1 site of the u-PAR cDNA. The following sequence depicts the carboxy-terminal region of the normal u-PAR:

Detailed Description Text (137):
which codes for a u-PAR molecule ending with Arg Leu and thus missing the last 8 amino acids (mutant p-u-PAR-PFLM-1). This clone has been deposited as plasmid DNA in the Deutsche Sammlung von Mikroorganismen, Mascheroder Weg 1b, D-3300 Braunschweig, Federal Republic of Germany, on Mar. 27, 1990, in accordance with the provisions of

the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, and has received Accession No. DSM 5865.

Detailed Description Text (138):
p-u-PAR-PFLM-1 clone has been transfected into LB6 cells as described above and its expression compared with that of wildtype p-u-PAR-1 cDNA. As shown in FIG. 11, this mutant expresses a u-PAR molecule that is partly recovered in the medium and partly retained in the cells. In fact, cross-linking to iodinated ATF shows a single band in the medium and two bands in the Triton X-114 extract (prepared as described in Example 1). The lower molecular weight band corresponds to a molecular weight of the non-glycosylated u-PAR. Only the high molecular weight band is present on the cell surface (see below). The data presented in FIG. 11 indicate that approximately 10 times as much protein is present in the medium with respect to what is retained in the cell.

Detailed Description Text (139):
A second mutant has been prepared in which the carboxy-terminal 36 amino acids have been deleted from the u-PA receptor, thus leaving a protein with no trans-membrane and no glycolipid anchor domain. To obtain this mutant, oligonucleotide-directed mutagenesis was employed, using the system commercially available from Amersham, to insert a single EcoRV site. To this end, the following oligonucleotide was used which hybridizes to the nucleotides 935-952 of the u-PAR cDNA sequence:

Detailed Description Text (147):
Acetylcholinesterases from human and bovine erythrocytes, phospholipase A.sub.2 from bee venom and myelin basic protein from bovine brain were from Sigma. Phospholipase D from cabbage and phosphatidylinositol-specific phospholipase C from Bacillus cereus (PI-PLC) were from Boehringer Mannheim. u-PAR was purified from PMA-stimulated U937 cells as in Example 1. Active human u-PA was purchaged from Serono and was DFP-inactivated as described (Nielsen et al., 1988); the amino terminal fragment (ATF) of u-PA was a kind gift from Dr. G. Cassani (LePetit, Italy). ATF, u-PAR and DFP-inhibited u-PA were radio-labelled as described (Nielsen et al., 1988) except that 0.1% (v/v) Triton X-100 was replaced by 0.1% (w/v) CHAPS in the case of u-PAR and by 0.01% (v/v) Tween 80 in the case of ATF and DFP-u-PA. Preparation of polyclonal rabbit antibodies against human u-PAR was carried out as described in Example 11.

Detailed Description Text (151):
Cell culture was performed as described in Example 1. Prior to metabolic labelling human U937 cells (5.times.10.sup.7 cells/dish) were PMA-stimulated (150 nM) for 5 hours in order to increase expression of u-PAR. For labelling with [.sup.3 H]ethanolamine and [.sup.3 H]myristic acid the cells were cultured in RPMI 1640 medium, while labelling with myo-[.sup.3 H]inositol was performed in Eagle's minimum essential medium. Both media were supplemented with: 2 mM L-glutamine, 5 mM Na-pyruvate, 200 units/ml penicillin, 25 .mu.g/ml streptomycin, 25 mM HEPES (pH 7.4), 0.5 mg/ml defatted BSA and 4.times. normal concentration of non-essential amino acids. All tracers were added from stock solutions in 25 mg/ml defatted BSA, 0.1 M HEPES (pH 7.4) to a final concentration of 0.1 mCi/ml in 10 ml media and metabolic labelling was allowed to proceed for 15 hours at 37.degree. C. Subsequently, the adherent cells were acid treated, washed and lyzed with 5 ml ice-cold 1% precondensed Triton X-114, 0.1 M Tris (pH 8.1), 10 .mu.g/ml Trasylol, 1 mM PMSF and 0.2 mM ZnCl.sub.2. Finally, detergent-phase separation was performed as described in Example 1.

Detailed Description Text (154):
Tricine-SDS-PAGE and Amino Acid Analysis

Detailed Description Text (156):
The Coomassie stained u-PAR was prepared for amino acid analysis by acid hydrolysis directly on the excised PVDF-membrane at 110.degree. C. in 100 .mu.l of redistilled 6M HCl including 0.05% (w/v) phenol and 5 .mu.l of 1% (w/v) DTDPA in 2 M NaOH as published (Ploug et al., 1989). Amino acid analysis was performed on a Waters amino acid analyzer, equipped with o-phtaldialdehyde derivatization essentially as described (Barkholt and Jensen, 1989). However, the chromatographic system was

modified slightly to increase resolution of basic amino acids. Elution was still performed by a pH-gradient resulting from mixing two non-halide buffers A and B (for composition see Barkholt and Jensen, 1989), but the gradient consisted of the following linear segments: initial eluant 100% A, 88% A and 12% B at 15 min, 60% A and 40% B at 24 min, 55% A and 45% B at 26 min, 50% A and 50% B at 36 min, 30% A and 70 B at 40 min, 25% A and 75% B at 64 min, 100% A at 65 min and 100% A from 65 to 70 min.

Detailed Description Text (158):
SDS-PAGE, chemical cross-linking with disuccinimyl suberate (DSS) and an analytical detergent phase separation was performed with Triton X-114 as described in Example 1.

Detailed Description Text (161):
Amino Acid Analysis of Purified u-PAR

Detailed Description Text (162):
Amino. acid analysis of the purified u-PAR (see Example 1) revealed the presence of an unidentified compound in the acid hydrolysate that reacted with o-phtaldialdehyde and eluted just after ammonia during cation-exhange chromatography (FIGS. 12A-12B). A similar peak was observed when u-PAR was purified from non-stimulated U937 cells (2.times.10.sup.10 cells), but otherwise treated identically (data not shown). This unknown compound behaved as a covalent constituent of u-PAR, as it persisted within the purified protein despite boiling it in 2% SDS followed by Tricine-SDS-PAGE and electroblotting onto a 0.45 .mu.m polyvinylidene diflouride (PVDF) membrane in the presence of 10% (v/v) MeOH. Furthermore, the compound was a specific constituent of the Coomassie stained u-PAR, as it was absent, when appropriate pieces of PVDF-membranes just above and below the protein stained area were excised and prepared for amino acid analysis by the same procedure (FIG. 12B). In addition, several stained proteins and peptides previously analyzed by this approach did not reveal the presence of this particular component (Ploug et al., 1989).

Detailed Description Text (163):
For amino acid analysis in this study, a special gradient was designed for the cation-exchange chromatography that allowed an increased resolution of common as well as various uncommon, basic amino acids without impairing reproducibility of their retention times (see Materials and Methods section). By this method the unidentified compound in u-PAR reproducibly eluted after 55.3 min, between ammonia (53.5 min) and arginine (60.8 min). As various physiological occurring arginine derivatives are expected to possess approx. similar retention times, several methylated arginine derivatives were tested, including: N.sup.w,N.sup.w -dimethylarginine (53.8 min), N.sup.w, N'.sup.w -dimethylarginine (54.4 min) and N.sup.w -monomethylarginine (58.6 min). None of these retention times were in agreement with the one observed for the unidentified compound in u-PAR. However, when authentic ethanolamine was tested, it showed exactly the same retention time as that for the unidentified compound. Furthermore, upon hydrolysis of both human and bovine erythrocyte acetylcholinesterases, a compound with this retention time was also observed, whereas it was absent in the hydrolysate from e.g. myelin basic protein. Acetylcholinesterases isolated from erythrocytes contain ethanolamine as a covalent constituent in a glycolipid membrane anchor, while myelin basic protein posseses a partly methylated arginine residue. It is therefore concluded that u-PAR does contain ethanolamine, covalently linked to the protein by acid labile bonds (e.g. ester or amide bonds). Quantitative analysis of the data in FIG. 12 shows that each u-PAR molecule contains 2-3 ethanolamine residues (see also Table 5).

Detailed Description Text (171):
When purified u-PAR was subjected to detergent-phase separation by Triton X-114, it almost quantitatively partitioned into the detergent phase, as assessed by cross-linking to, .sup.125 I-labelled ATF (FIG. 15A), thus demonstrating the very hydrophobic properties of the receptor. Incubation with PI-PLC altered the hydrophobicity of the u-PA binding protein substantially, as more than 50% of the ATF-binding activity was now recovered in the aqueous phase (FIG. 15B). It proved impossible to achieve a higher level of this conversion in the purified u-PAR preparation by increasing the concentration of PI-PLC. These data are in accordance with the fraction of cell associated u-PA which had been released in the previous

experiment by PI-PLC treatment of intact PMA-stimulated U937 cells (FIG. 13). This finding may indicate that a partial resistance (approx. 50%) against bacterial PI-PLC is a genuine feature of the u-PAR population in vivo. Other phospholipases (PLD and PLA.sub.2) did not induce any significant change in the hydrophobic properties of the purified u-PAR (FIG. 15C).

Detailed Description Text (176):
Apart from demonstrating the presence of approx. 2 mol ethanolamine/mol u-PAR (FIGS. 12A-B and Table 5), amino acid analysis revealed additional information about potential post-translational processing of this membrane receptor. When the calculated amino acid composition for the purified u-PAR was compared with that predicted for the nascent protein from cDNA sequence, several reproducible and significant discrepancies arose (Table 5). In particular, the actual determinations of Ala and Leu were too low, whereas those of Tyr and Phe were too high (Table 5). Interestingly, however, it was possible to bring the calculated and the predicted amino acid compositions into perfect agreement provided that the last 29-31 COOH-terminal residues were removed during some posttranslational event (Table 5). Thus, on the basis of the determined amino acid composition and the accuracy/precision normally obtained for this equipment, it is assumed that there exists a COOH-terminal processing site in u-PAR. According to this model, processing is expected to occur at one of the residues Ser.sub.282, Gly.sub.283 or Ala.sub.284 --as indicated in FIG. 16.

Detailed Description Text (193):
The effect of PMA on production of u-PAR protein was studied by cross-linking experiment. .sup.125 I-labelled aminoterminal fragment (ATF) of the urokinase were chemically cross linked to the detergent phase of phase-separated Triton X-114 extracts prepared from U937 cells treated with PMA for different time periods. FIG. 18 shows a weak signal of .sup.125 I-ATF cross-linked to the u-PAR in control U937 cells. After increasing time of PMA treatment both an increase in the strength of signal and a change to a lower electrophoretic mobility was seen.

Detailed Description Text (215):
Human fibrosarcoma cells (HT-1080, CCL 121) were obtained from the American Type Culture Collection, Rockville, Md. Confluent cell layers were grown in plastic Linbro wells (2 cm.sup.2 ; Flow Laboratories) in Eagle's minimal essential medium (MEM) supplemented with 10% heat-inactivated (56.degree. C. for 60 minutes) fetal calf serum (Gibco), 100 IU/ml penicillin and 50 .mu.g/ml streptomycin. After reaching confluence, the cells were rinsed three times with MEM containing 0.2% bovine serum albumin (BSA), then changed to either serum-free medium (0.5 ml) or medium containing 10% heat-inactivated and plasminogen-depleted (i.e. absorbed with lysine-Sepharose; Pharmacia, Uppsala, Sweden) fetal calf serum as indicated in the Examples.

Detailed Description Text (217):
Human plasminogen (with glutamic acid N-terminal) was prepared by affinity chromatography on lysine-Sepharose (Deutsch, D. G., and E. T. Mertz, "Plasminogen: Purification from human plasma by affinity chromatography", Science 170: 1095-1097, 1970) from freshly separated, unfrozen human plasma pretreated with 10 .mu.M p-nitrophenyl guanidinobenzoate, 1 mM phenylmethylsulfonylfluoride and 0.1 .mu.g/ml of an anti-catalytic murine monoclonal IgG antibody to human t-PA (ESP-2; see MacGregor, I. R. et al., "Characterization of epitopes on human tissue plasminogen activator recognised by a group of monoclonal antibodies", Thromb. Haem. 53: 45-50, 1985); American Diagnostica, Greenwich, Conn.).

Detailed Description Text (218):
Inhibition studies made use of the following reagents added to cell cultures: an anti-catalytic murine monoclonal IgG antibody to human plasmin (anti-plg 1, 20 .mu.g/ml; see Sim, P-S. et al., "Monoclonal antibodies inhibitory to human plasmin: definitive demonstration of a role for plasmin in activating the proenzyme of urokinase-type plasminogen activator", Eur. J. Biochem. 158: 537-542, 1986); aprotinin (Trasylol, Bayer, Leverkusen, FRG; 200 KIU/ml); tranexamic acid (Cyclokapron, Kabi Vitrum, Stockholm; 10 .mu.M and 100 .mu.M); human type-2 plasminogen activator inhibitor minactivin (see Golder, J. P. et al., "Minactivin: A human monocyte product which specifically inactivates urokinase-type plasminogen

activators", Eur. J. Biochem. 136: 517-522, 1983), PAI-2 purified from cultures of human U-937 histiocytic lymphoma cells (see Leung, K-C. et al., "The resistance of fibrin-stimulated tissue plasminogen activator to inactivation by a class PAI-2 inhibitor (minactivin)", Thromb. Res. 46: 755-766, 1987) titration equivalent of 3.6 IU u-PA/ml; an anti-catalytic murine monoclonal IgG antibody to human u-PA (clone 2 (10 .mu.g/ml) in Nielsen, L. S. et al., "Enzyme-linked immunosorbent assay for human urokinase-type plasminogen activators and its proenzyme using a combination of monoclonal and polyclonal antibodies", J. Immunoassay 7: 209-228, 1986); the anti-catalytic monoclonal antibody to human t-PA (10 .mu.g/ml); a neutralising murine monoclonal IgG antibody to human PAI-1 (Nielsen, L. S. et al., "Monoclonal antibodies to human 54,000 molecular weight plasminogen activator inhibitor from fibrosarcoma cells--inhibitor neutralization and one-step affinity purification", Thromb. Haem. 55: 206-212, 1986) (10 .mu.g/ml) and diisopropyl fluorophosphate (DFP)-inactivated u-PA (0-10 .mu.g/ml).

Detailed Description Text (220):
Active two-chain u-PA (Ukidan, Serono) was dissolved in 0.1 M Tris-HCl, pH 8.1, 0.1% Tween 80 (Tris/Tween). A freshly prepared solution of 500 mM DFP (Sigma) in isopropanol was added to yield a final DFP concentration of 5 mM. After thorough mixing, the sample was incubated for 2 hours at 37.degree. C., after which period addition of DFP was repeated as above. After renewed incubation for 2 hours at 37.degree. C., the reaction was terminated by thorough dialysis at 0.degree. C. against Tris/Tween. No residual DFP inhibitor could be detected when the preparation was tested in an activity assay of soluble urokinase.

Detailed Description Text (224):
Cell culture supernatants were assayed for pro-u-PA and active u-PA by the following modification of an immunocapture method (Stephens et al., 1988; Stephens et al., 1987). Microtitre wells of polystyrene immunoplates (type 269620, A/S Nunc, Roskilde, Denmark) were coated overnight at 37.degree. C. with 50 .mu.l of a solution of goat IgG antibodies to human u-PA (cat. # 398, American Diagnostica). The coating solution contained 2.5 .mu.g of IgG per ml of 0.1 M sodium carbonate (pH 9.8). After rinsing, the wells were treated with conditioned medium (50 ml) for 2 hours at 23.degree. C., then rinsed again. Half the wells were then treated with 50 .mu.l of freshly prepared 2 .mu.M p-nitrophenyl guanidinobenzoate (NPGB, Sigma) (Dan.o slashed., K., and E. Reich, "Plasminogen activator from cells transformed by an oncogenic virus--Inhibitors of the activator reaction", Biochim. Biophys. Acta 566: 138-151, 1979) for 20 minutes at 37.degree. C. The other half (controls) received 50 .mu.l of rinsing buffer (0.05% Tween 20 in PBS). After rinsing, u-PA was assayed in all the wells by addition of 40 .mu.l of plasminogen solution (100 .mu.g/ml in assay buffer consisting of 50 mM sodium glycinate (pH 7.8), 0.1% Triton X-100, 0.1% gelatin and 10 mM 6-aminocaproic acid which also contained a very low concentration of plasmin (10 ng/ml)), and incubation took place for 30 minutes at 37.degree. C. This concentration of plasmin in the plasminogen incubation was sufficient to enable full realization of the potential activity of pro-u-PA (cf. Petersen et al., 1988). The plasmin produced by this incubation was assayed by its thioesterase activity (Green, G. D. G., and E. Shaw, "Thiobenzyl benzyloxycarbonyl-L-lysinate, substrate for a sensitive colorimetric assay for trypsin-like enzymes", Anal. Biochem. 93: 223-226, 1979) by the addition of 200 .mu.l of a solution containing 200 mM potassium phosphate (pH 7.5), 200 mM KCl, 0.1% Triton X-100, 220 .mu.M Z-lysine thiobenzyl ester (Peninsula Laboratories, Belmont, Calif.) and 220 .mu.M 5,5'-dithiobis(2-nitrobenzoic acid) (Sigma). This mixture was incubated for 30 minutes at 37.degree. C., and the absorbancies of the wells were read at 405 nm. Active u-PA (60,000 IU/mg) was purchased from Calbiochem-Behring (La Jolla, Calif.) and pro-u-PA (potential activity 90,000 IU/mg) was obtained from American Diagnostica.

Detailed Description Text (225):
Pro-u-PA and active u-PA bound to the cell layer were recovered for immunocapture assays by the same method as was used in the metabolic labelling (see above). Each culture well (2 cm.sup.2) was eluted with 150 .mu.l of acid glycine at pH 3 (Stoppelli et al., 1986). For conditioned medium and cell-bound u-PA, the u-PA activity assayed after NPGB treatment was expressed as a percentage of the total activity obtained without NPGB treatment, and this percentage used as an index of pro-u-PA content (pro-u-PA index). The conditions used for the NPGB treatment were

previously established (Stephens et al., 1988) to allow selective inactivation of active u-PA, while leaving the pro-u-PA unchanged and still able to be activated by the added plasmin to the same extent as untreated pro-u-PA.

Detailed Description Text (228):
Plasmin bound to the cell layer was recovered and assayed as follows. After harvest of culture medium, the cells were rinsed three times with PBS (plasmin assays of further rinses were negative); then the bound plasmin was specifically eluted (Miles, L. A., and E. F. Plow, "Binding and activation of plasminogen on the platelet surface", J. Biol. Chem. 260: 4303-4311, 1985) with a solution of 1 mM tranexamic acid in the same rinsing solution (150 .mu.l/well). Plasmin activity was assayed in eluate samples (50 .mu.l) as above with an incubation time of 3 hours at 37.degree. C. Tranexamic acid at 1 mM had no effect on the thioesterase activity of plasmin in these assays.

Detailed Description Text (231):
After addition of purified preparations of human plasminogen to cultures of human fibrosarcoma cells (HT-1080) growing in a medium with 10% plasminogen-depleted fetal calf serum, plasmin activity could be recovered as a bound fraction from the cell layer. Upon varying the concentration of added plasminogen, the bound plasmin activity increased in a dose-dependent manner (FIG. 22). The binding was specific so that after rinsing of the cells with isotonic buffer, the plasmin could be released by 1 mM tranexamic acid. This agent disrupts interactions with plasminogen or plasmin which involve the lysine affinity sites of the heavy-chain kringles (Miles, supra). The plasmin released from HT-1080 cell surfaces was conveniently measured by its thioesterase activity, a method which was unaffected by the presence of tranexamic acid. Some plasmin activity was also detected in the medium. At a concentration of 40 .mu.g/ml human plasminogen added to 0.5 ml of medium above a confluent 2 cm.sup.2 cell layer, activity corresponding to 28 ng of plasmin could be recovered from the cell layer with tranexamic acid, while 10 ng was measurable in the medium after 3 hours of incubation at 37.degree. C. This concentration of plasminogen is well below the 200 .mu.g/ml present in normal human plasma.

Detailed Description Text (234):
Incubation of cells carrying plasmin with fresh serum-free medium showed that approximately 40% of the activity remained bound after 2 hours at 37.degree. C. (FIGS. 24A and 24B). When the cells were incubated in 10% serum-containing medium, the same fraction (40%) of this activity could be recovered from the cells; the bound plasmin was not inactivated by the serum. However, only about 11% (compared to 60% for serum-free medium) could be detected in the serum-containing medium (FIG. 24B). When 1 mM tranexamic acid was added to the serum-containing medium, no plasmin activity could be recovered from the cells (FIG. 24A).

Detailed Description Text (245):
To prevent the interference of PAI-1, the neutralizing PAI-1 antibody was therefore included in the next experiment in which the effect of the plasmin inhibitor aprotinin and the effect of an anti-catalytic monoclonal antibody to human plasmin on the conversion of pro-u-PA to active u-PA were studied. As shown in Table 7, both these inhibitors increased the relative amount of pro-u-PA, thus demonstrating that the activation of cell-bound pro-u-PA was catalyzed by plasmin. To study whether this was an effect of cell-bound plasmin, the effect of tranexamic acid in a concentration of 100 .mu.M was also tested, which concentration completely inhibits binding of plasmin to the cells, but does not affect the ability of plasmin to activate pro-u-PA in solution (R. Stephens, unpublished results). This treatment markedly decreased the relative amount of active u-PA, indicating that the activation of the cell surface pro-u-PA is catalyzed by the surface-bound plasmin.

Detailed Description Text (246):
The following additions were made to cell layers growing in MEM medium (0.5 ml) containing 10% heat-inactivated and plasminogen-depleted fetal calf serum: native human plasminogen (Plg, 40 .mu.g/ml); anti-catalytic monoclonal antibody to human u-PA (10 .mu.g/ml); anti-catalytic monoclonal antibody to human t-PA (10 .mu.g/ml); PAI-2 (titration equivalent of 3.6 UI u-PA/ml); anti-catalytic monoclonal antibody to human plasmin (20 .mu.g/ml); aprotinin (200 KIU/ml); tranexamic acid (TA, as shown). The cultures were incubated for the times shown before assay of cell-bound

plasmin. The incubation with plasminogen was used as the 100% control for bound plasmin.

Detailed Description Text (248):
Confluent cell layers were incubated for 2 hours at 37.degree. C. with MEM medium (0.5 ml) containing 10% heat-inactivated and plasminogen-depleted fetal calf serum with the following additions: native human plasminogen (Plg, 40 .mu.g/ml); neutralizing monoclonal antibody to human PAI-1 (10 .mu.g/ml); aprotinin (200 KIU/ml); anti-catalytic monoclonal antibody to human plasmin (20 .mu.g/ml); and tranexamic acid (TA, as shown). Half the wells were then treated with aprotinin (200 KIU/ml) and used for assay of bound u-PA and its pro-u-PA index. The other half were used for elution and assay of bound plasmin.

Detailed Description Text (256):
Binding assay. Before binding, U937 cells were incubated for 1 hour at 4.degree. C. in RPMI 1640 medium supplemented with 0.1% bovine serum albumin and 50 mM Hepes (pH 7.4). The cells were then acid-treated in 50 mM glycine-HCl, 100 mM NaCl (pH 3) for 3 minutes at 4.degree. C. and quickly neutralized with half a volume of 0.5 M Hepes, 100 mM NaCl (pH 7.4). One million cells were then resuspended in 0.2 ml of binding buffer (phosphate buffered saline supplemented with 0.1% bovine serum albumin) containing iodinated ligands (about 50,000 cpm corresponding to 0.1 nM for ATF and 0.05 nM for pro-u-PA and u-PA) and incubated for the indicated time at 4.degree. C. After binding, the cells were centrifugated and washed with cold phosphate buffered saline--0.1% bovine serum albumin. Non-specific binding was determined in the presence of 100 nM unlabelled u-PA.

Detailed Description Text (258):
Amidolytic assay. u-PA activity was assayed by incubating 100 .mu.l aliquots of binding mixtures or supernatants of binding assays in 0.05 M Tris-HCl (pH 7.5), 40 mM NaCl, 0.01% Tween 80, with 1 mM of the plasmin-specific substrate S2390 (Kabi Vitrum, Sweden) and 0.5 .mu.M plasminogen in a final volume of 0.3 ml. The time dependence of the colour development was measured following the absorbance at 405 nm (Petersen et al., 1988).

Detailed Description Text (270):
Surprisingly, in the absence of PAI-1 in the binding mixture, two weaker bands with molecular weights of about 69 and 90 kD are detected. This background was dependent on the presence of the cells and could not be eliminated by different pretreatment of the cells. These bands were not retained on Sepharose 4B columns coupled with anti-PAI-1 IgG. This is in contrast to the complexes found on cells after incubation with preformed PAI-1/u-PA complexes which, as expected, could be isolated from the acid washes of cells by immunoaffinity chromatography (data not shown). This is in agreement with the very low levels of PAI-1 in U937 cells (Lund et al., 1988). The nature of the two contaminating bands, therefore, remains unknown and will require further investigation. They may represent complexes of receptor-bound u-PA with PAI-2 (Genton et al., 1987) or with protease nexin-1 (Baker et al., Cell 21: 37-47, 1980).

Detailed Description Text (286):
Unlike the internalization of the nexin-protease complexes which are formed in solution and subsequently bind to the cells and are internalized via so far uncharacterized receptors (Baker et al., 1980), the u-PA:PAI-1 complex is bound to the receptor itself (see Example 8) and subsequently undergoes internalization and degradation. This receptor, therefore, must alternate between two possible configurations: one in which it binds active u-PA and in which it dictates plasminogen activation on the cell surface; and another in which it binds the inhibited enzyme and in which it favours internalization and degradation of the ligand. This property could be exploited for internalizing toxins and thus specifically kill the cells that express the u-PA receptor, or by forcing the state of the receptor from one state (i.e. exposed) to another, through PAI-1 or PAI-1 analogues.

Detailed Description Text (290):
Plasminogen was purified from fresh human plasma as previously described (Dan.o slashed. and Reich, 1979), and was further separated into its two isoforms by

elution from lysine-Sepharose with a linear gradient of 6-amino-hexanoic acid. Plasminogen isoform 2 was used in all experiments described here. u-PA (M.sub.r 55,000) was obtained either by plasmin activation of pro-uPA (Ellis et al., 1987) or as Ukidan (Serono). Both preparations were greater than 95% high molecular weight u-PA by SDS-polyacrylamide gel electrophoresis. The concentration of active u-PA in these preparations was determined by active-site titration with p-nitrophenyl-p-guanidinobenzoate (Sigma Chem. Co.). DFP-inactivated u-PA was prepared as described in Example 1. The murine monoclonal antibody to u-PA was clone 2 from Nielsen et al., 1986. Active PAI-1 was purified from the serum-free conditioned medium of Hep G2 cells by affinity chromatography on immobilized anhydro-urokinase (Wun et al., 1989). PAI-2 was purified from U937 cell lysates by chromatofocusing as described (Kruithof et al., 1986). The concentrations of active inhibitor in the various PAI preparations were determined by titration against u-PA immediately before use in the kinetic experiments. PAI-1 or PAI-2 at varying concentrations between 1 nM and 100 nM were incubated with active-site titrated u-PA (20 .mu.M) for 1 hour at 37.degree. C. in 0.05 M Tris, 0.1 M NaCl pH 7.4 containing 0.2% bovine serum albumin. Residual u-PA activity was then measured by hydrolysis of 0.2 mM Glu-Gly-Arg-AMC (Bachem, Switzerland).

Detailed Description Text (351):
2) Reaction buffer: 0.1% bovine serum albumin+0.1% Triton X-100 in PBS (0.1% BSA, 0.1% Triton X-100/PBS).

Detailed Description Text (372):
6) Washing buffer: PBS+0.1% Tween 20, pH 7.4 (PBS/Tween 20).

Detailed Description Text (393):
5) PBS+0.1% Tween 20, pH 7.4 (PBS/Tween 20).

Detailed Description Text (415):
Samples of purified human u-PA receptor (Example 1) were subjected to SDS-polyacrylamide gel electrophoresis under non-reducing conditions on a 6-16% gradient gel. By the use of fluorescent molecular weight markers run in neighbouring lanes, the electrophoretic region corresponding to the antigen was excised. The gel piece was lyophilized and subsequently macerated in a Mikro-Dismembrator II apparatus (B. Braun AG, Federal Republic of Germany). The polyacrylamide powder was reconstituted in Tris-buffered saline, mixed with Freund's incomplete adjuvant and used for injection of a New Zealand white rabbit. The animal received 5 injections, each containing approximately 3 .mu.g of the antigen, over a 10 week period, followed by a single 8 .mu.g injection after an additional 7 weeks. Serum was drawn 1 week after the last injection, and IgG was prepared by Protein A-Sepharose chromatography. In order to remove antibodies against trace impurities in the injected antigen, the antibody was absorbed by consecutive passages through columns containing immobilized human u-PA and the protein mixture constituting the Triton X-114 detergent phase from PMA-stimulated U937 cells (see Example 1), respectively. The antibody preparation obtained did not inhibit the amidolytic or plasminogen activator activity of u-PA in solution.

Detailed Description Text (418):
Western Blotting--Samples of affinity purified u-PAR or detergent phase from Triton X-114 extracts of PMA-stimulated U937 cells were subjected to SDS-PAGE under reducing conditions on 6-16% gradient gels. The gels were electroblotted onto nitrocellulose sheets. The sheets were rinsed and blocked with 30% fetal calf serum in Tris-buffered saline, pH 7.4. The sheets were incubated with mouse anti-u-PAR serum or control serum (i.e. mouse antiserum against porcine mucins), diluted in fetal calf serum in Tris-buffered saline. The sheets were rinsed, incubated with secondary antibody (alkaline phosphatase-conjugated rabbit anti-mouse Ig (Dakopatts, Copenhagen)), and developed with nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate/Levamisol.

Detailed Description Text (426):
Rabbit polyclonal antibodies were prepared by immunizing a rabbit with polyacrylamide gel material containing affinity-purified u-PAR that had subsequently been subjected to preparative SDS-PAGE. The IgG fraction was isolated from the obtained antiserum and absorbed by passage through columns with immobilized human

u-PA and immobilized membrane-protein mixture derived from PMA-stimulated U937 cells, respectively. The antibody recognized u-PAR in the Triton X-114 detergent phase from PMA-stimulated U937 cells (FIG. 46A). Thus, a protein in the 50-65 kD range was recognized (lanes 1 and 2) which could be identified as being u-PAR by the ability to form a 100-110 kD conjugate with DFP-treated u-PA after the performance of chemical cross-linking (see Example 1 for methods) (lane 3). No staining was obtained with DFP-treated u-PA alone (lanes 5 and 6), and the cross-linking procedure did not alter the electrophoretic appearance of u-PAR when no DFP-treated u-PA was added (lane 2). In none of the samples was any band stained with the pre-immune IgG from the same rabbit, prepared in the same manner (FIG. 46B).

Detailed Description Text (431):
u-PA was dialyzed overnight against 0.1 M Na.sub.2 HCO.sub.3 with 0.1% Triton X-100. N-biotin-hydroxysuccinimide was dissolved in N,N-dimethylformamide (5 mM). To the u-PA preparation was added 0.1 .mu.l of this solution per .mu.g of u-PA, and the reaction was allowed to run for 1 hour at room temperature. Excess labelling compound was removed by dialysis overnight against 0.1 M NaHPO.sub.4, pH 8.0, with 0.5 M NaCl and 0.1% Triton X-100.

Detailed Description Text (432):
Cultured cells (PMA-treated U937) or cryostat sections of freshly frozen human chorion were treated for 3 minutes at room temperature with 0.05 M glycine, pH 3.0 with 0.1 M NaCl, neutralized with 0.5 M HEPES, pH 7.5 with 0.1 M NaCl and incubated at 4.degree. C. with 200 nM of biotinylated DFP-treated u-PA dissolved in PBS with 0.1% BSA (PBS-BSA). Competition experiments were performed by simultaneous incubation with biotinylated DFP-treated u-PA (200 nM) and purified unlabelled u-PA (2 .mu.M).

Detailed Description Text (440):
Both assays were carried out in microtiter plates, using chromogenic substrates (see below), the cleavage of which was followed by measuring the absorbance at 405 nm in an ELISA reader. Proteolysis buffer (0.1 M Tris/HCl, pH 8.1, 0.1% Triton X-100) was used as the reaction buffer and for the dilution of all samples. Affinity purified u-PAR (see Example 1) was added as indicated or substituted by a protein devoid sample of the same buffer compositon. Materials and methods not specified below were those described by Petersen et al. (1988). All samples were analysed in triplicate.

Detailed Description Text (442):
Human 54 kDa two-chain u-PA (Ukidan, Serono) was preincubated with u-PAR or buffer at the concentrations indicated for 15 min at room temperature. Plasminogen (10 .mu.g/ml final concentration) and H-D-Valyl-L-leucyl-L-lysine-p-nitroanilide dihydrochloride (Kabi product S-2251), termed substrate S1 below (final concentration 400 .mu.M) were added in a final reaction volume of 250 .mu.l, and cleavage of the substrate was followed during incubation at 37.degree. C. Standard curves were drawn from assays of the following final concentrations of u-PA: 8, 16, 32, 64, 128 and 256 pg/ml.

Detailed Description Text (444):
Human pro-u-PA was preincubated with u-PAR or buffer for 10 min at room temperature. Plasmin (10 ng/ml final concentration) was added and the samples were incubated at 37.degree. C. Aliquots were taken after the following periods of incubation: 1, 2, 5, 10, 20, 30 and 60 min. After the periods indicated, plasmin activity within each sample was stopped by the addition of Trasylol (10 .mu.g/ml final concentration). Each aliquot was assayed for u-PA amidolytic activity by addition of 400 .mu.M (final concentration) of L-Pyroglutamyl-glycyl-L-arginine-p-nitronanilide hydrochloride (Kabi product (S-2444; termed substrate S2 below) in a final reaction volume of 200 .mu.l, followed by incubation at 37.degree. C. and absorbance measurement. The absorbance values were compared to a standard curve obtained with known concentrations of 54 kDa two-chain u-PA (Ukidan, Serono) in the same assay of amidolytic activity, performed simultaneously and using the same buffer composition.

Detailed Description Text (454):
Samples of purified u-PAR were treated with PI-PLC (500-fold final dilution of the Boehringer Mannheim preparation) for 30 min at 37.degree. C. This treatment led to

an approx. 50% delipidation of u-PAR as judged by the shift of the ATF cross-linking activity towards the buffer phase in the Triton X114 phase separation system (see Example 1).

Detailed Description Text (460):
Appella E, Robinson E A, Ullrich S J, Stoppelli M P, Corti A, Cassani G, Blasi F (1987) The receptor-binding sequence of urokinase. A biological function for the growth-factor module of proteases. J Biol Chem 262: 4437-4440

Detailed Description Text (465):
Barkholt V, Jensen A L (1989) Amino acid analysis: Determination of cysteine plus half-cysteine in proteins after hydrochloric acid hydrolysis with a disulfide compound as additive. Anal Biochem 177: 318-322

Detailed Description Text (472):
Bordier C (1981) Phase Separation of integral membrane proteins in Triton X-114 solution. J Biol Chem 256: 1604-1607

Detailed Description Text (488):
Eaton D L, Scott R W, Baker J B (1984) Purification of human fibroblast urokinase proenzyme and analysis of its regulation by proteases and protease nexin. J Biol Chem 259: 6241-6247

Detailed Description Text (505):
Hopp T P, Woods K R (1981) Prodiction of protein antigenic determinants from amino acid sequences. Proc. Natl. Acad. Sci. USA 78: 3824-3828

Detailed Description Text (531):
Miles L A, Plow E F (1986) Topography of the high-affinity lysine binding site of plasminogen as defined with a specific antibody probe. Biochemistry 25: 6926-6933

Detailed Description Text (533):
Morrissey J H, Falhrai H, Edgington T S (1987) Molecular cloning of the cDNA for tissue factor, the cellular receptor for the initiation of the coagulation protease cascade. Cell 50: 129-135

Detailed Description Text (536):
Nelles L, Lijnen H R, Collen D, Holmes W E (1987) Characterization of recombinant human single chain urokinase-type plasminogen activator mutants produced by site-specific mutagenesis of lysine 158. J Biol Chem 262: 5682-5689

Detailed Description Text (547):
Ploug M, Jensen A L, Barkholt V (1989) Determination of amino acid compositions and NH.sub.2 -terminal sequences of peptides electroblotted onto PVDF membranes from tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis: Application to peptide mapping of human complement component C3. Anal Biochem 181: 33-39.

Detailed Description Paragraph Table (1):
TABLE 1 Amino acid composition of affinity purified u-PAR, determined after Tricine-SDS-PAGE, electroblotting onto a PVDF membrane, and alkylation Asp/Asn 33.2 Thr.sup.a 21.4 Ser.sup.b 26.3 Glu/Gln.sup.c 43.2 Pro 11.4 Gly 28.2 Ala 8.4 Cys (as Cys (Cm)) 28.4 Val 11.9 Met.sup.d 7.7 Ile 6.7 Leu 26.5 Tyr 8.0 Phe 5.7 His 12.8 Lys 11.1 Arg 20.0 Glucosamine.sup.e 30.8 .sup.a Corrected for a 5% loss during hydrolysis. .sup.b Corrected for a 10% loss during hydrolysis. .sup.c Slight overestimation possible, due to formation of pyro-glutamic acid in amino acid standard mixture. .sup.d Corrected for a 30% loss normally observed during electrophoresis and blotting (35). .sup.e Corrected for a 50% loss during hydrolysis.

Detailed Description Paragraph Table (2):
TABLE 2 N-terminal amino acid sequence of u-PAR. Parentheses indicate an identification classified as tentative. Question mark indicates no identification. Where footnotes are present, they indicate the best guess. A. Direct sequencing of affinity purified u-PAR after dialysis against 0.1 M acetic acid and lyophilization. The initial yield was 70 pmol PTH-Leu at step 1. Note that direct sequencing does

not allow the identification of cysteine residues. Res. no. 1 2 3 4 5 6 7 8 9 10 Amino acid Leu ? ? Met Gln ?.sup.a Lys Thr Asn Gly residue Res. no. 11 12 13 14 15 16 Amino acid Asp ? Arg Val (Glu) Glu (SEQ ID NO:1) residue B. Sequence obtained after Tricine-SDS-PAGE, electroblotting and alkylation. The PVDF membrane contained 35 pmol u-PAR, as estimated from a parallel amino acid analysis experiment (Table 1). The initial yield was 19.5 pmol PTH-Leu at step 1. The repetitive yield, based on Leu 1, Leu 19 and Leu 23, was 96%. Cys indicates the identification of the PTH derivative of carboxymethyl cycteine in the alkylated protein. Res. no. 1 2 3 4 5 6 7 8 9 10 Amino acid Leu ? Cys Met Gln Cys Lys Thr Asn Gly residue Res. no. 11 12 13 14 15 16 17 18 19 20 Amino acid Asp Cys (Arg) Val Glu Glu (His) Ala Leu Gly residue Res. no. 21 22 23 24 25 26 27 28 29 30 Amino acid Gln ?.sup.b Leu ?.sup.c (Arg) Thr (Thr) Ile Val ?.sup.d (SEQ ID NO:2) residue .sup.a Asn? .sup.b Asp? .sup.c Arg/Cys? .sup.d Arg/Thr?

Detailed Description Paragraph Table (3):
TABLE 3 The amino acid sequences of the N-terminal peptide and the derived synthentic oligonucleotide. Amino acid sequence: (SEQ ID NO:24) Leu ... ... Met Gln Asn Lys Thr Asn Gly Asp Derived oligonucleotide: (SEQ ID NO:28) 5' ATG CAA AAT AAA ACX AAT GGX GAT 3' G C G C Synthesized probe: (SEQ ID NO:29) 5' ATC ICC ATT IGT CTT ATT CTG CAT 3' G C G C T G T The hybridization conditions used for this probe were 5x SSC and 50.degree. C.

Detailed Description Paragraph Table (8):
TABLE 5 Amino acid composition of purified u-Par compared with that deduced from its cDNA before and after the proposed COOH-terminal processing.sup.a Amino Predicted Determined after acid from cDNA acid hydrolysis SD A) Entire u-PAR sequence (Leu.sub.1 -Thr.sub.313) Asp + Asn 29 32.7 0.5 Thr.sup.b 25 21.9 0.5 Ser.sup.b 25 25.8 0.5 Glu + Gln.sup.c 37 41.8 1.3 Pro 12 11.1 0.3 Gly 29 29.4 1.1 Ala 11 8.3 0.1 Cys.sup.d 28 28.8 1.0 Val 12 12.1 0.2 Met 7 6.0 0.6 Ile 8 6.7 0.1 Leu 31 26.9 0.7 Tyr 7 7.8 0.2 Phe 5 5.7 0.1 His 13 12.8 0.1 Lys 10 10.8 0.2 Arg 20 20.3 0.2 Trp 4 nd nd Ethanolamine -- 2.6 0.4 B) Assumed u-PAR sequence after processing (Leu.sub.1 -Ala.sub.284) Asp + Asn 29 29.8 0.4 Thr.sup.b 20 20.0 0.5 Ser.sup.b 24 23.6 0.4 Glu + Gln.sup.c 36 38.1 1.2 Pro 9 10.2 0.3 Gly 26 26.8 1.0 Ala 8 7.6 0.1 Cys.sup.d 28 26.3 0.9 Val 12 11.0 0.2 Met 6 5.5 0.5 Ile 7 6.1 0.1 Leu 24 24.5 0.6 Tyr 7 7.1 0.1 Phe 5 5.2 0.1 His 12 11.6 0.1 Lys 10 9.9 0.2 Arg 19 18.6 0.2 Trp 2 nd nd Ethanolamine -- 2.4 0.4 Footnotes to Table 5 .sup.a Purified u-PAR was prepared for amino acid analysis as described in the legend to FIGS. 12A-B. The presented values represent the average of 3 independent determinations. The data were normalized relative to all amino acids, except trytophan, assuming a total number of 309 residues for the nascent u-PAR and 282 for the fully processed protein (omitting 4 and 2 tryptophan residues, respectively). Amino acid numbering was based upon the cDNA sequence for u-PAR without the signal # sequence (Example 3). .sup.b The values for these hydroxyamino acids were corrected for decomposition during hydrolysis - Ser (5%) and Thr (10%). .sup.c A slight overestimation is expected due to the formation of pyroglutamic acid in the amino acid standard mixture. .sup.d In one sample cysteine was derivatized before hydrolysis by in situ alkylation using iodoacetamide and subsequently quantified as S-carboxymethylcysteine after acid hydrolysis. In general, the yield of this alkylation procedure is 95% (Ploug, 1989). Otherwise, cysteine was derivatized during hydrolysis in the presence of 3,3'-dithiodipropionic acid (DTDPA) and quantified as the mixed disulfide compound (Cys-x) formed between cysteine and DTDPA. .sup.e nd = not determined. .sup.f SD = standard deviation (absolute number of residues). The results in this Example unequivocally demonstrate that u-PAR has a glycosyl-phosphotidylinositol anchor and is C-terminally processed.

Detailed Description Paragraph Table (15):
SEQUENCE LISTING (1) GENERAL INFORMATION: (iii) NUMBER OF SEQUENCES: 32 (2) INFORMATION FOR SEQ ID NO: 1: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 16 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1 Leu Xaa Xaa Met Gln Xaa Lys Thr Asn Gly Asp Xaa Arg Val Glu Glu 1 5 10 15 (2) INFORMATION FOR SEQ ID NO: 2: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2 Leu Xaa Cys Met Gln Cys Lys Thr Asn Gly Asp Cys

Arg Val Glu Glu 1 5 10 15 His Ala Leu Gly Gln Xaa Leu Xaa Arg Thr Thr Ile Val Xaa 20 25 30 (2) INFORMATION FOR SEQ ID NO: 3: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 92 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3 Leu Arg Cys Met Gln Cys Lys Thr Asn Gly Asp Cys Arg Val Glu Glu 1 5 10 15 Cys Ala Leu Gly Gln Asp Leu Cys Arg Thr Thr Ile Val Arg Leu Trp 20 25 30 Glu Glu Gly Glu Glu Leu Glu Leu Val Glu Lys Ser Cys Thr His Ser 35 40 45 Glu Lys Thr Asn Arg Thr Leu Ser Tyr Arg Thr Gly Leu Lys Ile Thr 50 55 60 Ser Leu Thr Glu Val Val Cys Gly Leu Asp Leu Cys Asn Gln Gly Asn 65 70 75 80 Ser Gly Arg Ala Val Thr Tyr Ser Arg Ser Arg Tyr 85 90 (2) INFORMATION FOR SEQ ID NO: 4: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 99 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4 Leu Glu Cys Ile Ser Cys Gly Ser Ser Asp Met Ser Cys Glu Arg Gly 1 5 10 15 Arg His Gln Ser Leu Gln Cys Arg Ser Pro Glu Glu Gln Cys Leu Asp 20 25 30 Val Val Thr His Trp Ile Gln Glu Gly Glu Glu Gly Arg Pro Lys Asp 35 40 45 Asp Arg His Leu Arg Gly Cys Gly Tyr Leu Pro Gly Cys Pro Gly Ser 50 55 60 Asn Gly Phe His Asn Asn Asp Thr Phe His Phe Leu Lys Cys Cys Asn 65 70 75 80 Thr Thr Lys Cys Asn Glu Gly Pro Ile Leu Glu Leu Glu Asn Leu Pro 85 90 95 Gln Asn Gly (2) INFORMATION FOR SEQ ID NO: 5: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 90 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5 Arg Gln Cys Tyr Ser Cys Lys Gly Asn Ser Thr His Gly Cys Ser Ser 1 5 10 15 Glu Glu Thr Phe Leu Ile Asp Cys Arg Gly Pro Met Asn Gln Cys Leu 20 25 30 Val Ala Thr Gly Thr His Glu Pro Lys Asn Gln Ser Tyr Met Val Arg 35 40 45 Gly Cys Ala Thr Ala Ser Met Cys Gln His Ala His Leu Gly Asp Ala 50 55 60 Phe Ser Met Asn His Ile Asp Val Ser Cys Cys Thr Lys Ser Gly Cys 65 70 75 80 Asn His Pro Asp Leu Asp Val Gln Tyr Arg 85 90 (2) INFORMATION FOR SEQ ID NO: 6: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 36 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6 GCCAGACTGT GGGGAGGCAC TCTCCTCTGG ACCTAA 36 (2) INFORMATION FOR SEQ ID NO: 7: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 11 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7 Ala Arg Leu Trp Gly Gly Thr Leu Leu Trp Thr 1 5 10 (2) INFORMATION FOR SEQ ID NO: 8: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 11 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (genomic) (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8 CCANNNNNTG G 11 (2) INFORMATION FOR SEQ ID NO: 9: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9 AGAGT 5 (2) INFORMATION FOR SEQ ID NO: 10: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10 ACAGT 5 (2) INFORMATION FOR SEQ ID NO: 11: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11 AGACT 5 (2) INFORMATION FOR SEQ ID NO: 12: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12 ACTGT 5 (2) INFORMATION FOR SEQ ID NO: 13: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 14 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13 CTAGTCTAGA CTAG 14 (2) INFORMATION FOR SEQ ID NO: 14: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 24 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14 AGACTCTAGT CTAGACTAGA CTGT 24 (2) INFORMATION FOR SEQ ID NO: 15: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 17 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15 GACCTGGATA TCCAGTA 17 (2) INFORMATION FOR SEQ ID NO: 16: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 25 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE

DESCRIPTION: SEQ ID NO:16 Glu Pro Gly Ala Ala Thr Leu Lys Ser Val Ala Leu Pro Phe Ala Ile 1 5 10 15 Ala Ala Ala Ala Leu Val Ala Ala Phe 20 25 (2) INFORMATION FOR SEQ ID NO: 17: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 26 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17 Cys Lys Asp Ser Ser Ile Leu Val Thr Lys Lys Phe Ala Leu Thr Val 1 5 10 15 Val Ser Ala Ala Phe Val Ala Leu Leu Phe 20 25 (2) INFORMATION FOR SEQ ID NO: 18: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 32 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18 Thr Thr Asp Ala Ala His Pro Gly Arg Ser Val Val Pro Ala Leu Leu 1 5 10 15 Pro Leu Leu Ala Gly Thr Leu Leu Leu Glu Thr Ala Thr Ala Pro 20 25 30 (2) INFORMATION FOR SEQ ID NO: 19: (i) SEQUENCE CHARACTERISTICS:

Detailed Description Paragraph Table (16):
(A) LENGTH: 29 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19 Val Ser Ala Ser Gly Thr Ser Pro Gly Leu Ser Ala Gly Ala Thr Val 1 5 10 15 Gly Ile Met Ile Gly Val Leu Val Gly Val Ala Leu Ile 20 25 (2) INFORMATION FOR SEQ ID NO: 20: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 34 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20 Val Lys Cys Gly Gly Ile Ser Leu Leu Val Gln Asn Thr Ser Trp Leu 1 5 10 15 Leu Leu Leu Leu Leu Ser Leu Ser Phe Leu Gln Ala Thr Asp Phe Ile 20 25 30 Ser Leu (2) INFORMATION FOR SEQ ID NO: 21: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 34 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21 Tyr Arg Ser Gly Ala Ala Pro Gln Pro Gly Pro Ala His Leu Ser Leu 1 5 10 15 Thr Ile Thr Leu Leu Met Thr Ala Arg Leu Trp Gly Gly Thr Leu Leu 20 25 30 Trp Thr (2) INFORMATION FOR SEQ ID NO: 22: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 1400 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 47..1054 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22 AGAGAAGACG TGCAGGGACC CCGCGCACAG GAGCTGCCCT CGCGAC ATG GGT CAC 55 Met Gly His 1 CCG CCG CTG CTG CCG CTG CTG CTG CTG CTC CAC ACC TGC GTC CCA GCC 103 Pro Pro Leu Leu Pro Leu Leu Leu Leu Leu His Thr Cys Val Pro Ala 5 10 15 TCT TGG GGC CTG CGG TGC ATG CAG TGT AAG ACC AAC GGG GAT TGC CGT 151 Ser Trp Gly Leu Arg Cys Met Gln Cys Lys Thr Asn Gly Asp Cys Arg 20 25 30 35 GTG GAA GAG TGC GCC CTG GGA CAG GAC CTC TGC AGG ACC ACG ATC GTG 199 Val Glu Glu Cys Ala Leu Gly Gln Asp Leu Cys Arg Thr Thr Ile Val 40 45 50 CGC TTG TGG GAA GAA GGA GAA GAG CTG GAG CTG GTG GAG AAA AGC TGT 247 Arg Leu Trp Glu Glu Gly Glu Glu Leu Glu Leu Val Glu Lys Ser Cys 55 60 65 ACC CAC TCA GAG AAG ACC AAC AGG ACC CTG AGC TAT CGG ACT GGC TTG 295 Thr His Ser Glu Lys Thr Asn Arg Thr Leu Ser Tyr Arg Thr Gly Leu 70 75 80 AAG ATC ACC AGC CTT ACC GAG GTT GTG TGT GGG TTA GAC TTG TGC AAC 343 Lys Ile Thr Ser Leu Thr Glu Val Val Cys Gly Leu Asp Leu Cys Asn 85 90 95 CAG GGC AAC TCT GGC CGG GCT GTC ACC TAT TCC CGA AGC CGT TAC CTC 391 Gln Gly Asn Ser Gly Arg Ala Val Thr Tyr Ser Arg Ser Arg Tyr Leu 100 105 110 115 GAA TGC ATT TCC TGT GGC TCA TCA GAC ATG AGC TGT GAG AGG GGC CGG 439 Glu Cys Ile Ser Cys Gly Ser Ser Asp Met Ser Cys Glu Arg Gly Arg 120 125 130 CAC CAG AGC CTG CAG TGC CGC AGC CCT GAA GAA CAG TGC CTG GAT GTG 487 His Gln Ser Leu Gln Cys Arg Ser Pro Glu Glu Gln Cys Leu Asp Val 135 140 145 GTG ACC CAC TGG ATC CAG GAA GGT GAA GAA GGG CGT CCA AAG GAT GAC 535 Val Thr His Trp Ile Gln Glu Gly Glu Glu Gly Arg Pro Lys Asp Asp 150 155 160 CGC CAC CTC CGT GGC TGT GGC TAC CTT CCC GGC TGC CCG GGC TCC AAT 583 Arg His Leu Arg Gly Cys Gly Tyr Leu Pro Gly Cys Pro Gly Ser Asn 165 170 175 GGT TTC CAC AAC AAC GAC ACC TTC CAC TTC CTG AAA TGC TGC AAC ACC 631 Gly Phe His Asn Asn Asp Thr Phe His Phe Leu Lys Cys Cys Asn Thr 180 185 190 195 ACC AAA TGC AAC GAG GGC CCA ATC CTG GAG CTT GAA AAT CTG CCG CAG 679 Thr Lys Cys Asn Glu Gly Pro Ile Leu Glu Leu Glu Asn Leu Pro Gln 200 205 210 AAT GGC CGC CAG TGT TAC AGC TGC AAG GGG AAC AGC ACC CAT GGA TGC 727 Asn Gly Arg Gln Cys Tyr Ser Cys Lys Gly Asn Ser Thr His Gly Cys 215 220 225 TCC TCT GAA GAG ACT TTC CTC ATT GAC TGC CGA GGC CCC ATG AAT CAA 775 Ser Ser Glu Glu Thr Phe Leu Ile Asp Cys Arg Gly Pro Met Asn Gln 230 235 240 TGT CTG GTA GCC ACC GGC ACT CAC GAA CCG AAA AAC CAA AGC TAT ATG 823 Cys Leu Val Ala Thr Gly Thr His Glu Pro Lys Asn Gln Ser Tyr Met 245 250 255 GTA AGA GGC TGT GCA ACC GCC TCA ATG TGC CAA CAT GCC CAC CTG GGT 871 Val Arg Gly Cys Ala Thr Ala Ser Met Cys Gln His Ala His Leu Gly 260 265 270 275 GAC GCC TTC AGC ATG AAC CAC ATT GAT GTC TCC TGC TGT ACT AAA AGT 919 Asp Ala Phe Ser Met Asn His Ile Asp Val Ser Cys Cys Thr Lys

Ser 280 285 290 GGC TGT AAC CAC CCA GAC CTG GAT GTC CAG TAC CGC AGT GGG GCT GCT 967
Gly Cys Asn His Pro Asp Leu Asp Val Gln Tyr Arg Ser Gly Ala Ala 295 300 305 CCT CAG
CCT GGC CCT GCC CAT CTC AGC CTC ACC ATC ACC CTG CTA ATG 1015 Pro Gln Pro Gly Pro Ala
His Leu Ser Leu Thr Ile Thr Leu Leu Met 310 315 320 ACT GCC AGA CTG TGG GGA GGC ACT
CTC CTC TGG ACC TAAACCTGAA 1061 Thr Ala Arg Leu Trp Gly Gly Thr Leu Leu Trp Thr 325
330 335 ATCCCCCTCT CTGCCCTGGC TGGATCCGGG GGACCCCTTT GCCCTTCCCT CGGCTCCCAG 1121
CCCTACAGAC TTGCTGTGTG ACCTCAGGCC AGTGTGCCGA CCTCTCTGGG CCTCAGTTTT 1181 CCCAGCTATG
AAAACAGCTA TCTCACAAAG TTGTGTGAAG CAGAAGAGAA AAGCTGGAGG 1241 AAGGCCGTGG GCAATGGGAG
AGCTCTTGTT ATTATTAATA TTGTTGCCGC TGTTGTGTTG 1301 TTGTTATTAA TTAATATTCA TATTATTTAT
TTTATACTTA CATAAAGATT TTGTACCAGT 1361 GGAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAA
1400 (2) INFORMATION FOR SEQ ID NO: 23: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH:
335 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE:
protein (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ
ID NO:23 Met Gly His Pro Pro Leu Leu Pro Leu Leu Leu Leu His Thr Cys 1 5 10 15
Val Pro Ala Ser Trp Gly Leu Arg Cys Met Gln Cys Lys Thr Asn Gly 20 25 30 Asp Cys Arg
Val Glu Glu Cys Ala Leu Gly Gln Asp Leu Cys Arg Thr 35 40 45 Thr Ile Val Arg Leu Trp
Glu Glu Gly Glu Glu Leu Glu Leu Val Glu 50 55 60 Lys Ser Cys Thr His Ser Glu Lys Thr
Asn Arg Thr Leu Ser Tyr Arg 65 70 75 80 Thr Gly Leu Lys Ile Thr Ser Leu Thr Glu Val
Val Cys Gly Leu Asp 85 90 95 Leu Cys Asn Gln Gly Asn Ser Gly Arg Ala Val Thr Tyr Ser
Arg Ser 100 105 110 Arg Tyr Leu Glu Cys Ile Ser Cys Gly Ser Ser Asp Met Ser Cys Glu
115 120 125 Arg Gly Arg His Gln Ser Leu Gln Cys Arg Ser Pro Glu Glu Gln Cys 130 135
140 Leu Asp Val Val Thr His Trp Ile Gln Glu Gly Glu Glu Gly Arg Pro 145 150 155 160
Lys Asp Asp Arg His Leu Arg Gly Cys Gly Tyr Leu Pro Gly Cys Pro 165 170 175 Gly Ser
Asn Gly Phe His Asn Asn Asp Thr Phe His Phe Leu Lys Cys 180 185 190 Cys Asn Thr Thr
Lys Cys Asn Glu Gly Pro Ile Leu Glu Leu Glu Asn 195 200 205 Leu Pro Gln Asn Gly Arg
Gln Cys Tyr Ser Cys Lys Gly Asn Ser Thr 210 215 220 His Gly Cys Ser Ser Glu Glu Thr
Phe Leu Ile Asp Cys Arg Gly Pro 225 230 235 240 Met Asn Gln Cys Leu Val Ala Thr Gly
Thr His Glu Pro Lys Asn Gln 245 250 255 Ser Tyr Met Val Arg Gly Cys Ala Thr Ala Ser
Met Cys Gln His Ala 260 265 270 His Leu Gly Asp Ala Phe Ser Met Asn His Ile Asp Val
Ser Cys Cys 275 280 285 Thr Lys Ser Gly Cys Asn His Pro Asp Leu Asp Val Gln Tyr Arg
Ser 290 295 300 Gly Ala Ala Pro Gln Pro Gly Pro Ala His Leu Ser Leu Thr Ile Thr 305
310 315 320 Leu Leu Met Thr Ala Arg Leu Trp Gly Gly Thr Leu Leu Trp Thr 325 330 335
(2) INFORMATION FOR SEQ ID NO: 24: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 11
amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii)
MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE
DESCRIPTION: SEQ ID NO:24 Leu Xaa Xaa Met Gln Asn Lys Thr Asn Gly Asp 1 5 10 (2)
INFORMATION FOR SEQ ID NO: 25: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 24 base
pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii)
MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (ix) FEATURE: (A)
NAME/KEY: misc_feature (D) OTHER INFORMATION: /note= "N at positions 15 and 21
stands for the modified nucleotide x (3-(3-amino-3-carboxypropyl)uridine,(acp3)u)"
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25 ATGCAGAATA AGACNAATGG NGAY 24 (2)
INFORMATION FOR SEQ ID NO: 26: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 25 base
pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii)
MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (ix) FEATURE: (A)
NAME/KEY: misc_feature (D) OTHER INFORMATION: /note= "N at positions 4 and 11 stands
for modified nucleotide base i (inosine)" (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26
RTCNCCATRT NGTCTTATTC TGCAT 25 (2) INFORMATION FOR SEQ ID NO: 27: (i) SEQUENCE
CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (C) STRANDEDNESS:
single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A)
ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27 Gly Arg Gly Asp Ser 1 5
(2) INFORMATION FOR SEQ ID NO: 28: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 24 base
pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii)
MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (ix) FEATURE: (A)
NAME/KEY: misc_feature (D) OTHER INFORMATION: /note= "N at positions 15 and 21
stands for the modified nucleotide x (3-(3-amino-3-carboxypropyl)uridine,(acp3)u)"
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28 ATGCAAAATA AAACNAATGG NGAT 24 (2)
INFORMATION FOR SEQ ID NO: 29: (i) SEQUENCE CHARACTERISTICS:

Detailed Description Paragraph Table (17):
(A) LENGTH: 24 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D)
TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM:
unknown (ix) FEATURE: (A) NAME/KEY: misc_feature (D) OTHER INFORMATION: /note= "N at
positions 4 and 10 stands for modified nucleotide base i (inosine)" (xi) SEQUENCE
DESCRIPTION: SEQ ID NO:29 ATCNCCATTN GTCTTATTCT GCAT 24 (2) INFORMATION FOR SEQ ID

NO: 30: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30 TGGTGATATG AAGGAGAGAA 20 (2) INFORMATION FOR SEQ ID NO: 31: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 19 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31 CAGTGGATGT TGCCTTTAC 19 (2) INFORMATION FOR SEQ ID NO: 32: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 21 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: unknown (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32 Asp Cys Leu Asn Gly Gly Thr Cys Val Ser Asn Lys Tyr Phe Ser Asn 1 5 10 15 Ile His Trp Cys Asn 20

Other Reference Publication (23):
Nelles, L., et al., "Characterization of Recombinant Human Single Chain Urokinase-type Plasminogen Activator Mutants Produced by Site-specific Mutagenesis of Lysine 158," J. Biol. Chem., 262(12):5682-89, (1987).*

CLAIMS:

15. The method of claim 2 in which the substance comprises an amino acid sequence which (a) is identical to SEQ ID NO:32, or (b) differs from SEQ ID NO:32 by not more than five substitutions, insertions, or deletions of amino acids.

16. The method of claim 2 in which the substance comprises an amino acid sequence which (a) is identical to SEQIDNO:32, or (b) differs from SEQ ID NO:32 by not more than five substitutions of amino acids.

17. The method of claim 16 in which the substitutions of amino acids, if any, were conservative substitutions.

L7: Entry 5 of 12 File: USPT Dec 26, 2000

DOCUMENT-IDENTIFIER: US 6165500 A
TITLE: Preparation for the application of agents in mini-droplets

Drawing Description Text (17):
FIG. 17 is a graphical representation of the blood glucose level data described in Example 236;

Drawing Description Text (18):
FIG. 18 is a graphical representation of the blood glucose level data described in Example 237;

Drawing Description Text (19):
FIG. 19 and FIG. 20 are graphical representations of the data pertaining to glucose depletion in blood, described in Example 238.

Detailed Description Text (31):
Edge active solvents which can be used according to this invention include, furthermore, short-chain acyl-, alkyl-, alkenyl, hydroxyacyl-, alkenyloxy- as well as aryl derivatives of different acids and bases, such as acetic acid, formic acid, propionic acid, butenoic acid, pentenoic acid, etc. of many amino acids, benzoic acid, phosphoric- and sulphuric acid, of ammonia, purine, pyrimidine, etc., provided that they do not impair the chemical integrity of the carriers and the agent molecules to an inacceptable extent.

Detailed Description Text (40):
Further, very commonly used special forms of non-ionic edge active substances are sold under the trademark "TWEEN". The cyclic part of this substance class is frequently a sorbitol ring. Residues R.sub.1, R.sub.2, R.sub.3 and R.sub.4 are frequently of the alkoxy- or alkenoxy-, and even more commonly of the polyene-, polyoxyalkene-, such as polyoxyethylene-, polyalcohol-, such as polyglycol-, or polyether type. Some of these chains can be apolar, corresponding to e.g. an acyl-, alkyl-, alkenyl-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain with 8-24 carbon atoms. If none of residues R.sub.1, R.sub.2, R.sub.3 or R.sub.4 is apolar, one of the side-chains of a branched chain or one of the termini must be hydrophobic.

Detailed Description Text (41):
Chains in the substances of TWEEN type are very frequently of the polyoxyethylene class. They mainly contain one terminal hydrogen atom and more rarely a methoxy group. One of the polyoxyethylene chains, however, contains a hydrophobic residue which preferably corresponds to an acyl-, alkyl-, alkenyl-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain with 4-24, and in particular 12-18 carbon atoms.

Detailed Description Text (42):
Edge active substances which are sold under the trademark "TRITON" are also useful according to this invention.

Detailed Description Text (61):
Another important group of anionic edge active substances are the derivatives of cholic acid. Their basic formula reads ##STR4## here, R.sub.1 corresponds to a proton, an OH-- or a carbonyl group and R.sub.2 can be a derivative of taurine or

glycocoll, for example. Particularly suitable are various salts of cholic acid (bile acid, 3alpha, 7alpha, 12alpha-trihydroxy-5beta-cholane-24-oin-acid), deoxycholic acid (3alpha, 12alpha-dihydroxy-5beta-cholane-24-oin-acid), chenodeoxycholic acid, glycocholic acid (N-(3alpha, 7alpha, 12alpha-trihydroxy-24-oxycholane-24-yl-)glycine), deoxycholic acid, glycodeoxycholic acid (N-(3alpha, 12alpha-dihydroxy-24-oxycholane-24-yl-)glycine), glycochenodeoxycholic acid, glycolitocholic acid, glycoursodeoxycholic acid, litocholic acid, taurodeoxycholic acid, taurocholic acid (3alpha, 7alpha, 12alphatrihydroxy-5beta-cholan-24-oin-acid-N-(sulfoethyl)amide), taurochenodeoxycholic acid, tauroglycocholic acid, taurolitocholic acid, taurolitocholic acid-3-sulfate, tauroursodeoxycholic acid, ursocholanic acid, ursodeoxycholic acid (3alpha, 7beta-dihydroxy-5beta-cholanic acid), the most common counterions being sodium or potassium.

Detailed Description Text (65):
The basic formula of the phosphorus-containing anionic edge active substances is ##STR5## in which n is zero or one. One of the two side chains R.sub.1 and R.sub.2 contains hydrogen, a hydroxy group or a short chain alkyl residue; the other contains an alkyl-, alkenyl-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain (or an alkenyl-, alkoxy-, alkenyloxy- or acyloxy-residue) with 8-24 carbon atoms. The R.sub.3 residue, as a rule, corresponds to hydrogen or an alkyl chain with less than 5 carbon atoms. R.sub.4 can be an anionic oxygen or a hydroxy group; an alkyl chain with up to 8 C-atoms can also appear as well as another carbohydrate residue with up to 12 carbon atoms; if R.sub.1 as well as R.sub.2 are hydrogen and/or hydroxy groups, a steroid residue, a sugar derivative, a chain containing an amino group, etc., can also appear. Alkyl residues can also be substituted.

Detailed Description Text (104):
The following surfactants are especially useful for biological purposes: N,N-bis(3-D-glucon-amidopropyl)cholamide (BigCHAP), Bis(2-ethylhexyl)sodium-sulfosuccinate, cetyl-trimethyl-ammonium-bromide, 3-((cholamidopropyl)-dimethylammonio)-2-hydroxy-1-propane sulfonate (CHAPSO), 3-((cholamidopropyl)-dimethylammonio)-1-propane sulfonate (CHAPS), cholate-sodium salt, decaoxyethylene-dodecyl-ether (Genapol C-100), decaethylene-isotridecyl-ether (Genapol X-100), decanoyl-N-methyl-glucamide (MEGA-10), decyl-glucoside, decyl-maltoside, 3-(decyldimethylammonio)-propane-sulfonate (Zwittergent 3-10), deoxy-bigCHAP, deoxycholate, sodium salt, digitonin, 3-(dodecyldimethylammonio)-propane-sulfonate (Zwittergent 3-12), dodecyl-dimethyl-amine-oxide (EMPIGEN), dodecylmaltoside, dodecylsulfate, glyco-cholate, sodium salt, glycodeoxycholate, sodium salt, heptaethylene-glycol-octyl-phenylether (triton X-114), heptyl-glucoside, heptyl-thioglucoside, 3-(hexadecyldimethylammonio)-propane-sulfonate (Zwittergent 3-14), hexyl-glucoside, dodecyl-dimethyl-amine-oxide (Genaminox KC), N-dodecyl-N,N-dimethylglycine (Empigen BB), N-decyl-sulfobetaine (Zwittergent 3-10), N-dodecyl-sulfobetaine (Zwittergent 3-12), N-hexadecyl-sulfobetaine (Zwittergent 3-16), N-tetradecyl-sulfobetaine (Zwittergent 3-14), N-octylsulfobetaine (Zwittergent 3-08), nonaethylene-glycol-monododecyl-ether (THESIT), nonaethylene-glycol-octyl-phenol-ether (triton X-100), nonaethylene-glycol-octyl-phenyl-ether (NP-40, Nonidet P-40), nonaethylene-dodecyl-ether, nonanoyl-N-methylglucamide (MEGA-9), nonaoxyethylene-dodecyl-ether (Lubrol PX, Thesit), nonyl-glucoside, octaethylene-glycol-isotridecylether (Genapol X-080), octaethylene-dodecyl-ether, octanoyl-N-methyl-glucamide (MEGA-8), 3-(octyldimethylammonio)-propanesulfonate (Zwittergent 3-08), octyl-glucoside, octylthioglucoside, entadecaethylene-isotridecyl-ether (Genapol X-150), polyethylene-polypropylene-glycol (Pluronic F-127), polyoxyethylene-sorbitane-monolaurate (Tween 20), polyoxyethylene-sorbitane-monooleate (Tween 80), taurodeoxycholate-sodium salt, taurocholate-sodium salt, 3-(tetradecyldimethylammonio)-propane-sulfonate (Zwittergent 3-14), etc.

Detailed Description Text (105):
Particularly suitable for pharmacological purposes are: cetyl-trimethyl-ammonium-salts (such as hexadecyltrimethylammoniumbromide, trimethylhexadecylaminebromo-salt), cetylsulfate salts (such as Na-salt, Lanette E),

cholate salts (such as Na- and ammonium-form) decaoxyethylenedodecyl-ether (Genapol C-100), deoxycholate salts, dodecyldimethyl-amine-oxide (Genaminox KC, EMPIGEN), N-dodecyl-N,N-dimethylglycine (Empigen BB), 3-(hexadecyldimethylammonio)propane-sulfonate (Zwittergent 3-14), fatty acid salts and fatty alcohols, glyco-deoxycholate salts, laurylsulfate salts (sodium dodecylsulfate, Duponol C, SDS, Texapon K12), N-hexadecyl-sulfobetaine (Zwittergent 3-16), nonaethylene-glycol-octyl-phenyl-ether (NP-40, Nonidet P-40), nonaethylene-dodecyl-ether, octaethylene-glycol-isotridecyl-ether (Genapol X-080), octaethylene-dodecyl-ether, polyethylene glycol-20-sorbitane-monolaurate (Tween 20), polyethylene glycol-20-sorbitane-monostearate (Tween 60), polyethylene glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylenecetylstearylether (Cetomacrogo, Cremophor 0, Eumulgin, C 1000) polyhydroxyethylene-4-laurylether (Brij 30), polyhydroxyethylene-23-laurylether (Brij 35), polyhydroxyethylene-8-stearate (Myrj 45, Cremophor AP), polyhydroxyethylene-40-stearate (Myrj 52), polyhydroxyethylene-100-stearate (Myrj 59), polyethoxylated castor oil 40 (Cremophor EL), polyethoxylated hydrogenated castor oil (Cremophor RH 40, Cremophor RH 60) polyethoxylated plant oils (Lebrafils), sorbitane-monolaurate (Arlacel 20, Span 20), taurodeoxycholate salts, taurocholate salts, polyethylene glycol-20-sorbitane-palmitate (Tween 40), Myrj 49 and polyethylene glycol derivatives of ricinols, etc.

Detailed Description Text (125):
at least one substance with antifibrinolytic activity, such as aminocapronic acid or tranexamic acid.

Detailed Description Text (130):
at least one substance which is an inhibitor of biological activity, such as actinomycine C1, alpha-amanitine, ampicilline, aphidicoline, aprotinine, calmidazolium (R24571), calpaine-inhibitor I, calpaine-inhibitor II, castanospermine, chloroamphenicol, colcemide, cordycepine, cystatine, 2,3-dehydro-2-desoxy-n-acetyl-neuraminic acid, 1-desoxymannojirimycinehydrochloride, 1-desoxynojirimycine, diacylglycerolkinase-inhibitor, P1, P5-di(adenosine-5'-)-pentaphosphate, ebelactone A, ebelactone B, erythromycine, ethidiumbromide, N-hydroxyurea, hygromycine B, kanamycine sulfate, alpha2-macroglobuline, N-methyl-1-desoxynojirimycine, mitomycine C, myxothiazol, novobiocine, phalloidine, phenylmethylsulfonylfluoride, puromycine-dihydrochloride, rifampicine, staurosporine, streptomycine sulfate, streptozotocine, G-strophanthine, swainsonine, tetracycline-hydrochloride, trifluoperazine-dihydrochloride, tunicamycine, etc.; useful proteinase inhibitors are, for example, (4-amidinophenyl)methanesulfonylfluoride (APMSF), antipaine-dihydrochloride, antithrombine III, alpha-1-antitrypsine, aprotinine, bestatine, calpaine-inhibitor I, calpaine-inhibitor II, L-1-chloro-3-(4-tosylamido)-7-amino-2-heptanone-hydrochloride (TLCK), L-1-chloro-3-(4-tosylamido)-4-phenyl-2-butanone (TPCK), chymostatine, cystatine, 3,4-dichlorisocoumarin, E 64, selastatinal, hirudin, kallikrein-inhibitor (aprotinine) L-leucinthiol, leupeptine, pepstatine, phenylinethylsulfonylfluoride (PMSF), phosphoramidone, TLCK (tosyl-lysine-chloromethyl-ketone), TPCK (tosyl-phenylalanine-chloromethyl-ketone), trypsine-inhibitors, etc.;

Detailed Description Text (196):
A carbohydrate, normally, has a basic formula C.sub.x (H.sub.2 O).sub.y, e.g. in sugar, starch, cellulose, and, moreover, can be derivatised in many different ways.

Detailed Description Text (198):
An aldose with five carbon atoms (aldo-pentose, or simply pentose) is for example D-arabinose, D-lyxose, D-ribose or D-xylose.

Detailed Description Text (200):
An aldose with six carbon atoms (aldo-hexose, or simply hexose) is e.g. D-allose, D-altrose, D-galactose, D-glucose, D-mannose or D-talose. A ketose with six carbon atoms (or simply keto-hexose) is e.g. D-fructose, D-psicose, D-sorbose or D-tagatose.

Detailed Description Text (202):
A carbohydrate residue, moreover, can be a natural disaccharide residue, e.g. a

disaccharide residue consisting of two hexoses. Such a disaccharide residue arises, for example, through condensation of two aldoses, e.g. D-galactose or D-glucose, or one aldose, e.g. D-glucose and one ketose, e.g. fructose; disaccharides formed from two aldoses, such as lactose or maltose, are preferably conjugated to the phosphatidyl group through the hydroxy group, which is located in position 6- of the corresponding pyranosyl residue. A disaccharide formed from an aldose and a ketose, such as saccharose, is preferably conjugated through a hydroxyl-group in position 6- of the pyranosyl residue or in position 1- of the furanosyl residue.

Detailed Description Text (204):
A carbohydrate can result from a cleaving action, starting with one of the mentioned mono- or disaccharides, by a strong oxidation agent, such as periodic acid. Amongst the biologically most important or most active carbohydrates are e.g.
2-acetamido-N-(epsilon-amino-caproyl)-2-deoxy-beta-gluccopyranosylamine,
2-acetamido-2-amino-1,2-dideoxy-beta-glucopyranose,
2-acetamido-1-beta-(aspartamido)-1,2-dideoxyglucose,
2-acetamido-4,6-o-benzyliden-2-deoxybeta-glucopyranose, 2-acetamido-2-deoxyallose,
3-acetamido-3-deoxyallose,
2-acetamido-2-deoxy-3-o-(beta-galactopyranosyl)-galactopyranose,
2-acetamido-2-deoxy-4-o-([4-o-beta-galactopyranosyl-beta-galactopyranosyl]
-beta-galactopyranosyl)-glucopyranose,
2-acetamido-2-deoxy-3-o-(beta-galactopyranosyl)-alpha-glucopyranose,
6-o-(2-acetamido-2-deoxy-4-[beta-galactopyranosyl]-beta-glucopyranosyl)-ga
lactopyranose,
4-o-acetamido-2-deoxy-6-o-(beta-galacto-4-o-(6-o-[2-acetamido-2-deoxy-beta
-glucopyranosyl]-beta-galactopyranosyl)glucopyranose, 2-acetamido-2-deoxygalactose,
2-acetamido-2-deoxyglucose, 3-acetamido-3-deoxyglucose pyranose,
6-o-(2-acetamido-2-deoxy-beta-glucopyranosyl)-galactopyranose,
2-acetamido-2-deoxy-1-thio-beta-glucopyranose 3,4,6-triacetate, acetopyruvic acid,
N-acetylchondrosamine, N-acetylgalactosamine, N-acetylglucosamine,
N-acetyl-alpha-glucosamine 1-phosphate, N-acetylglucosamine 6-phosphate,
N-acetylglucosamine 3-sulfate, N-acetylglucosamine 6-sulfate, N-acetylheparine,
N-acetyllactosamine, N-acetyl-beta-mannosamine, N-acetylneuraminic acid,
N-acetyl-neuramine-lactose, 1-o-acetyl-2,3,5-tri-o-benzoyl-beta-ribofuranose,
trans-aconic acid, adenine-9-beta-arabino-furanoside, adenosine
5'-diphospho-glucose, adenosine 5'-diphosphomannose, adonite, adonitol, adonose,
agar, algin, alginic acid, beta-allose, alpha glycerophosphate, alpha ketoglutaric
acid, altrose, (-)-altrose,
p-amino-benzyl-1-thio-2-acetamido-2-deoxy-beta-glucopyranoside,
N-epsilon-aminocaproyl-beta-fucopyranosylamine,
N-epsilon-aminocaproyl-alpha-galactopyranosylamine, 2-amino-2-deoxygalactopyranose,
6-amino-6-deoxyglucopyranose, 1-amino-1-deoxy-beta-glucose,
6-aminohexyl-N-acetyl-beta-thioglucosaminide, 6-aminohexyl-1-thio-beta-gal
actopyranoside, 5-aminoimidazole-4-carboxamidoxime-1-beta-ribofuranosyl
3':5'-cyclo-monophosphate, delta-aminolevulinic acid,
p-aminophenyl-2-acetamido-2-deoxy-beta-glucopyranoside,
p-aminophenyl-2-acetamido-2-deoxy-1-thio-beta-glucopyranoside,
p-aminophenyl-alpha-fucopyranoside, p-aminophenyl-alpha-galactopyranoside,
p-aminophenyl-beta-galactopyranoside, p-aminophenyl-alpha-glucopyranoside,
p-aminophenyl-beta-glucopyranoside, c-aminophenyl-beta-glucuronide,
p-aminophenyl-1-thio-beta-glucuronide, p-aminophenyl-beta-lactopyranoside,
p-aminophenyl-alpha-mannopyranoside, p-aminophenyl-beta-thiofucopyranoside,
p-aminophenyl-1-thio-beta-galactopy ranoside,
p-aminophenyl-1-thio-beta-glucopyranoside, p-minophenyl-1-thio-beta-xylopyranoside,
p-aminophenyl-beta-xylopyranoside, 5-amino-1-(beta-ribofuranosyl)imidazole
4-carboxamide, amygdaline, n-amyl beta-glucopyranoside, amylopectine, amylose,
apigenine 7-o-hesperidoside, arabinitol, arabinocytidine,
9-beta-arabinofuranosyladenine, 1-beta-arabinofuranosylcytosin, arabinose, arabinose
5-phosphate, arabinosylcytosine, arabite, arabitol, arbutine, atp-ribose,
atractyloside, aurothioglucose, n-butyl
4-o-beta-galactopyranosyl-beta-glucopyranoside, calcium gluconate, calcium
heptagluconate, carboxyatractyloside, carboxymethylamylose, carboxymethylcellulose,
carboxyethylthioethyl-2-acetamido-2-deoxy-4-o-beta-galactopyransol-beta-gl
ucopyranoside, carboxyethylthioethyl
4-o-(4-o-[6-o-alpha-glucopyranosyl-alpha-glucopyranosyl]-alpha-glucopyrano

syl)-beta-glucopyranoside,
4-o-(4-o-[6-o-beta-D-galactopyranosyl-beta-D-galactopyranosyl]-D-glucopyra nose,
carrageenan, D(+)cellobiose, D(+)cellopentaose, D(+)cellotetraose, D(+)cellotriose,
cellulose, cellulose caprate, cellulose carbonate, chitin, chitobiose, chitosan,
chitotriose, alpha-chloroalose, beta-chloroalose,
6-chloro-6-deoxy-alpha-glucopyranose, chondroitin sulfate, chondrosamine,
chondrosine, chrysophanic acid, colominic acid, convallatoxin, alpha-cyclodextrine,
beta-cyclodextrine, cytidine 5'-diphosphoglucose, cytosine 1-beta-arabinofuranoside,
daunosamine, n-decyl-beta-glucopyranoside, 5-deoxyarabinose,
2-deoxy-2-fluoroglucose, 3-deoxy-3-fluoroglucose, 4-deoxy-4-fluoroglucose,
6-deoxygalacto pyranose, 2-deoxygalactose, 1-deoxyglucohex-1-eno-pyranose
tetrabenzoat, 2-deoxyglucose, 6-deoxyglucose, 2-deoxyglucose 6-phosphate,
1-deoxymannojerimycin, 6-deoxymannose, 1-deoxy-1-morpholinofructose,
1-deoxy-1-nitroalutol, 1-deoxy-1-nitroaltitol, 1-deoxy-1-nitrogalactitol,
1-deoxy-1-nitromannitol, 1-deoxy-1-nitrosorbitol, 1-deoxy-1-nitrotalitol,
deoxynojirimycine, 3-deoxy-erythro-pentose, 2-deoxy-6-phosphogluconic acid,
2-deoxyribose, 3-deoxyribose, 2-deoxy-alpha-ribose 1-phosphate, 2-deoxyribose
5-phosphate, 5-deoxyxylofuranose, dextran, dextransulfate, dextrine, dextrose,
diacetonefructose, diacetonemannitol, 3,4-di-o-acetyl-6-deoxyglucal,
di-o-acetylrhamnal, 2,3-diamino-2,3-dideoxy-alpha-glucose,
6,9-diamino-2-ethoxyacridine lactate, 1,3:4,6-di-o-benzylidene mannitol,
6,6'-dideoxy-6,6'-difluorotrehalose, digalactosyl diglyceride, digalacturonic acid,
(+)digitoxose, 6,7-dihydrocoumarin-9-glucoside, dihydroxyacetone, dihydroxyacetone
phosphate, dihydroxyfumaric acid, dihydroxymalic acid, dihydroxytartaric acid,
dihydrozeatinriboside, 2,3-diphosphoglycerolic acid, dithioerythritol,
dithiothreitol, n-dodecyl beta-glucopyranoside, n-dodecyl beta-maltoside, dulcitol,
elemi-gum, endotoxin, epifucose, erythritol, erythro-pentulose, erythrose, erythrose
4-phosphate, erythrulose, esculin, 17-beta-estradiol-3-glucuronide 17-sulfate,
estriole glucuronide, estron beta-glucuronide, ethodin, ethyl
4-o-beta-D-galactopyranosyl)-beta-D-glucopyranoside,
ethyl2-acetamido-4-o-(2-acetamido-2-deoxy-beta-glucopyranosyl)-6-o-(alpha-
fucopyranosyl)-2-deoxy-beta-glucopyranoside,
ethyl2-acetamido-2-deoxy-4-o-(4-o-alpha-galactopyranosyl-beta-galactopyran
osyl)-beta-glucopyranoside, ethyl cellulose ethylene glycol chitin, ethyl
4-o-(4-o-alpha-galacto-pyranosyl-beta-galactopyranosyl)-beta-glucopyranosi de, ethyl
4-o-beta-galactopyranosyl-beta-glucopyranoside, ethyl pyruvate, ethyl
beta-thioglucoside, etiocholane-3alpha-ol-17-on glucuronide, ficoll,
6-fluoro-6-deoxyglucose, franguloside, fraxin, fructosazine, beta-(-)fructose,
fructose-1,6-diphosphate, fructose-2,6-diphosphate, fructose-1-phosphate,
fructose-6-phosphate, fucoidan, fucose, alpha-(-)-fucose-1-phosphate, fucosylamine,
2'-fucosyllactose, 3-fucosyllactose, fumaric acid, galactal, galactitol,
galactopyranosylamine, 3-o-beta-galactopyranosyl-arabinose,
4-o-beta-galactopyranosyl-fructofuranose, 4-o-(4-o-beta-galactopyranosyl
beta-galactopyranosyl)-glucopyranose, 4-o-alpha-galactopyranosyl-galactopyranose,
6-o-beta-galactopyranosylgalactose, 4-o-(beta-galactopyranosyl)-alpha-mannopyranose,
alpha-galactopyranosyl 1-phosphate, galactopyranosyl-beta-thio-galactopyranoside,
(+)galactosamine, alpha-galactosamine 1-phosphate, alpha-galactose 1-phosphate,
galactose 6-phosphate, galactose 6-sulfate, 6-(alpha-galactosido)glucose,
galacturonic acid, beta-gentiobiose, glucan, glucitol, glucoheptonic acid,
glucoheptose, glucoheptulose, gluconate 6-phosphate, gluconic acid,
1-o-alpha-glucopyranosyl-beta-fructofuranoside, 6-o-alpha-glucopyranosylfr uctose,
1-o-alpha-glucopyranosyl-alpha-glucopyranoside,
4-o-beta-glucopyranosylglucopyranose,
4-o-(4-o-[6-o-alpha-glucopyranosyl-alpha-glucopyranosyl]-alpha-glucopyrano
syl)glucopyranose, (+)glucosamine, alpha-glucosamine 6-2,3-disulfate,
alpha-glucosamine 1-phosphate, glucosamine 6-phosphate, glucosamine 2-sulfate,
alpha-glucosamine 3-sulfate, glucosamine 6-sulfate, glucosaminic acid, glucose,
alpha-glucose 1,6-diphosphate, glucose 1-phosphate, glucose 6-phosphate, glucose
6-sulfate, glucuronamide, glucuronic acid, alpha-glucuronic acid 1-phosphate,
glyceraldehyde, glyceraldehyde 3-phosphate, glycerate 2,3-diphosphate, glycerate
3-phosphate, glyceralic acid, alpha-glycerophosphate, beta-glycerophosphate,
glycogen, glycolaldehyde, glycol chitosan, n-glycolylneuraminic acid, glycyric acid,
glyoxylic acid, guanosine, 5'-diphosphoglucose, gulose, gums (accroides, agar, arab,
carrageenan, damar, elemi, ghatti, guaiac, guar, karaya, locust bonne, mast,
pontianac, storax, tragacanth, xanthan), heparin and heparin-like substances

(mesoglycan, sulodexide, etc.), heptakis(2,3,6-tri-o-methyl)-beta-cyclodextrin,
heptanoyl-N-methylglucamide, n-heptyl beta-glucopyranoside, hesperidin,
n-hexyl-beta-glucopyranoside, hyaluronic acid, 16-alpha-hydroxyestronglucuronide,
16-beta-hydroxyestron glucuronide, hydroxyethyl starch,
hydroxypropylmethyl-cellulose, 8-hydroxyquinolin-beta-glucopyranoside,
8-hydroxyquinolin glucuronide, idose, (-)-idose, indole-3- lactic acid,
indoxyl-beta-glucoside, epi-inositol, myo-inositol, myo-inositol bisphosphate,
myo-inositol-1,2-cyl phosphate, scyllo-inositol, inositolhexaphosphate,
inositolhexasulfate, myo-insoitol 2-monophosphate, myo-inositol trisphosphate,
(q)-epi-inosose-2, scyllo-inosose, inulin, isomaltose, isomaltotriose, isosorbid
dinitrate, 11-ketoandrosterone beta-glucuronide, 2-ketogluconic acid, 5-ketogluconic
acid, alpha-ketopropionic acid, lactal, lactic acid, lactitol, lactobionic acid,
lacto-N-tetraose, lactose, alpha-lactose 1-phosphate, lactulose, laminaribiose,
laminnarine, levoglucosan, beta-levulose, lichenan, linamarine, lipopolysaccharides,
lithiumlactate, lividomycine A, lyxose, lyxosylamine, maltitol, maltoheptaose,
maltohexaose, maltooligosaccharide, maltopentaose, maltose, alpha-(+)maltose
1-phosphate, maltotetraose, maltotriose, malvidine-3,5-diglucoside, mandelonitril
beta-glucoside, mandelonitril glucuronic acid, mannan, mannit, mannitol, mannitol
1-phosphate, alpha-mannoheptitol, mannoheptulose,
3-c-alpha-mannopyranosyl-mannopyranose, alpha(+)mannopyranosyl-1-phosphate,
mannosamine, mannosan, mannose, A(+)mannose 1-phosphate, mannose 6-phosphate,
(+)melezitose, A(+)melibiose, mentholglucuronic acid,
2-(3'-methoxyphenyl)-N-acetylneuraminic acid, methyl
3-o-(2-acetamido-2-deoxy-beta-galactopyranosyl)-alpha-galactopyranoside, methyl
4-o-(3-o-[2-acetamido-2-deoxy-4-o-beta-galactopyranosyl
beta-glucopyranosyl]-beta-galactopyranosyl)-beta-glucopyranoside, methyl
2-acetamido-2-deoxy-beta-glucopyranoside,
methyl3-o-(2-acetamido-2-deoxy-beta-glucopyranosyl)-beta-galactopyranoside ,
methyl6-o-(2-acetamido)-2-deoxy-beta-glucopyranosyl)-alpha-mannopyranosid e, methyl
acosaminide, methyl alpha-altropyranoside,
methyl3-amino-3-deoxy-alpha-mannopyranoside, methyl beta-arabinopyranoside, methyl
4,6-o-benzylidene-2,3-di-o-toluenesulfonyl-alpha-galactopyranoside, methyl
4,6-o-benzylidene-2,3-di-o-p-toluenesulfonyl-alpha-gluco-pyranoside, methyl
cellulose, methyl alpha-daunosaminide, methyl6-deoxy-alpha-galactopyranoside, methyl
6-deoxy-beta-galactopyranoside, methyl 6-deoxy-alpha-glucopyranoside, methyl
6-deoxy-beta-glucopyranoside, methyl
3,6-di-o-(alpha-mannopyranosyl)-alpha-mannopyranoside,
1-o-methyl-alpha-galactopyranoside, 1-o-methyl-beta-galactopyranoside, methyl
3-o-alpha-galactopyranosyl-alpha-galactopyranoside,
methyl-3-o-beta-galactopyranosyl-beta-galactopyranoside,
4-o-(2-o-methyl-beta-galactopyranosyl)glucopyranose, methyl
4-o-beta-galactopyranosyl-beta-glucopyranoside,
methyl-4-o-(beta-galactopyranosyl-alpha-mannopyranoside, 5--5-methylgalacto
pyranose, methylgalactoside, n-methylglucamine, 3-o-methyl-alpha-glucopyranose,
1-o-methyl-alpha-glucopyranoside, 1-o-methyl-beta-glucopyranoside, alpha-methyl
glucoside, beta-methyl glucoside, methyl glycol chitosan,
methyl-alpha-mannopyranoside, methyl-2-o-alpha-mannopyranosyl-alpha-mannopyranoside,
methyl 3-o-alpha-mannopyranosyl-alpha-mannopyranoside,
methyl-4-o-alpha-mannopyranosyl-alpha-mannopyranoside, methyl
6-o-alpha-mannopyranosyl-alpha-mannopyranoside, methyl alpha-rhamnopyranoside,
methyl alpha-ribofuranoside, methyl beta-ribofuranoside, methylbeta-thiogalactoside,
methyl 2,3,5-tri-o-benzoyl-alpha-arabinofuranoside,
4-methylumbelliferyl2-acetamido-4,6-o-benzylidene-2-deoxy-beta-glucopyrano side,
4-methylumbelliferyl N-acetyl-beta-galactosaminide, 4-methylumbelliferyl
N-acetyl-alpha-glucosaminide, 4-methylumbelliferyl-N-acetyl-beta-glucosaminide,
4-methylumbelliferyl-alpha-arabinofuranoside,
4-methylum-belli-feryl-alpha-arabinopyranoside,
4-methylum-belliferyl-beta-cellobioside,
4-methylumbelliferyl-beta-n,n'-diacetylchitobioside, 4-methylumbelliferyl
alpha-fucoside, 4-methylumbelliferyl beta-fucoside, 4-methylumbelliferyl
alpha-galactopyranoside, 4-methylumbelliferyl beta-galactopyranoside,
4-methylumbelliferyl alpha-galacto-side, 4-methylumbelliferyl beta -glucopyranoside,
4-methylumbelliferyl alpha-glucoside, 4-methylumbelliferyl beta-glucoside,
4-methylumbelliferyl beta-glucuronide, 4-methylumbelliferyl beta-mannopyranoside,
4-methylum-belliferylbeta-n,n',n"-triacetylchitotriose,

4-methyl-umbelliferyl2,3,5-tri-o-benzyl-alpha-arabinofuranoside, 4-methylumbelliferyl beta-xyloside, methyl beta-xylopyranoside, 2-o-methylxylose, alpha-methylxyloside, beta-methylxyloside, metrizamide, 2'-monophosphoadenosine 5'-diphosphoribose, 2'-monophosphoinosine 5'-diphosphoribose, mucine, muraminic acid, naringine, sodium lactate, sodium polypectate, sodium pyruvate, neoagarobiose, neoagarohexaitol, neoagarohexaose, neoagarotetraose, beta-neocarrabiose, neocarrabiose 4/1-sulfate, neocarrahexaose(2/4,4/1,4/3,4/5)-tetrasulfate, neocarratetraose(4/1,4/3)-disulfate, neocarratetraose(4/1)-sulfate, neohesperidin, dihydrochalcon, neohesperidose, neuraminic acid, neuraminic acid beta-methylglycoside, neuramine-lactose, nigeran, nigerantetrasaccharide, nigerose, n-nonyl glucoside, n-nonylbeta-glucopyranoside, octadecylthio-ethyl 4-o-alpha-galactopyranosyl-beta-galactopyranoside, octadecylthioethyl 4-o-(4-o-[6-o-alpha-glucopyranosyl-alpha-glucopyranosyl]-alpha-glucopyranosyl)-beta-glucopyranoside, octanoyl n-methylglucamide, n-octyl alpha-glucopyranoside, n-octyl-beta-glucopyranoside, oxidised starch, pachyman, palatinose, panose, pentaerythritol, pentaerythritol diformal, 1,2,3,4,5-pentahydroxy, capronic acid, pentosanpolysulfate, perseitol, phenolphthalein glucuronic acid, phenolphthalein mono-beta-glucosiduron phenyl 2-acetamido-2-deoxy-alpha-galactopyranoside, phenyl2-acetamido-2-deoxy-alpha-glucopyranoside, alpha-phenyl-N-acetyl-glucosaminide, beta-phenyl N-acetyl-glucosaminide, phenylethyl beta-galactoside, phenyl beta-galactopyranoside, phenyl beta-galactoside, phenyl alpha-glucopyranoside, phenyl beta-gluco-pyranoside, phenyl alpha-glucoside, phenyl beta-glucoside, phenyl beta-glucuronide, beta-phenyllactic acid, phenyl alpha-mannopyranoside, beta-phenylpyruvic acid, phenyl beta-thiogalactopyranoside, phenyl beta-thiogalactoside, phospho(enol)pyruvate, (+)2-phosphoglyceric acid, (-)3-phosphoglyceric acid, phosphohydroxypyruvic acid, 5-phosphorylribose 1-pyrophosphate, phytic acid, poly-N-acetylglucosamine, polygalacturonic acid, polygalacturonic acid methyl ester, polypectate, sodium, polysaccharide, 5beta-pregnane-3alpha, 2oalpha-diol glucuronide, n-propyl4-o-beta-galactopyranosyl-beta-glucopyranoside, prunasine, psicose, pullulan, quinolyl-8beta-glucuronic acid, (+)raffinose, alpha-rhamnose, rhapontine, ribitol, ribonolacton, ribose, D-2-ribose, alpha-ribose 1-phosphate, ribose 2-phosphate, ribose 3-phosphate, ribose 5-phosphate, ribulose, ribulose-1,5-diphosphate, ribulose 6-phosphate,

Detailed Description Text (205):
saccharic acid, saccharolactic acid, saccharose, salicin, sarcolactic acid, schardingers-alpha-dextrine, schardingers-beta-dextrine, sedoheptulosan, sedoheptulose 1,7-diphosphate, sialic acid, sialyllactose, sinigrine, sorbitol, sorbitol 6-phosphate, (+)-sorbose, (-)sorbose, stachyose, starch, storax, styrax, sucrose, sucrose monocaprate, tagatose, alpha-talose, (-)-talose, tartaric acid, testosterone-beta-glucuronide, 2,3,4,6-tetra-o-methyl-glucopyranose, thiodiglucoside, 1-thio-beta-galactopyranose, beta-thioglucose, 5-thioglucose, 5-thioglucose 6-phosphate, threitol, threose, (+)threose, (-)threose, thymidine 5'-diphosphoglucose, thymin 1-beta-arabinofuranoside, tragacanth, (+)trehalose, trifluorothymin, deoxyriboside, 3,3',5-trihydroxy-4'-methoxy-stilbene-3-o-beta-gluco-side, trimethylsilyl(+)arabinose, trimethylsilyldulcitol, trimethylsilyl-beta(-)fructose, trimethylsilyl(+)galactose, trimethylsilyl-alpha-(+)-glucose, trimethyl-silyl(+)mannitol, trimethylsilyl(+]rhamnose, trimethyl-silyl(-)sorbitol, trimethylsilyl(+)xylose, rac-1-o-tritylglycerol, (+)turanose, n-undecyl beta-gluco-pyranoside, uracil beta-arabinofuranoside, uridine 5'-diphospho-N-acetylglucosamine, uridine 5'-diphospho-galactose, uridine 5'-diphosphoglucose, uridine 5'-diphospho-glucuronic acid, uridine 5'-diphosphomannose, uridine 5'-diphosphoxylose, vancomycine, xanthan gum, xylane, xylite, xylitol, xylobiose, alpha-xylopyranosyl 1-phosphate, xylose, alpha-xylose 1-phosphate, xylose 5-phosphate, xylotriose, xylulose, xylulose 5-phosphate, yacca, zeatine riboside, zinclactate, zymosan A, etc.

Detailed Description Text (208):
Nucleotides, which can be effectively transported with the aid of transfersomes, encompass adenine, adenosine, adenosine-3',5'-cyclic monophosphate, N6,O2'-dibutyryl, adenosine-3',5'-cyclic monophosphate, N6,O2'-dioctanoyl, adenosine, n6-cyclohexyl, salts of adenosine-5'-diphosphate,

adenosine-5'-monophosphoric acid, adenosine-5'-o-(3-thiotriphosphate), salts of adenosine-5'-triphosphate, 9-beta-D-arabinoturanosyladenine, 1-beta-D-arabinoturanosylcytosine, 9-beta-D-arabinoturanosylguanine, 9-beta-D-arabinoturanosylguanine 5'-triphosphate, 1-beta-D-arabinoturanosylthymine, 5-azacytidine, 8-azaguanine, 3'-azido-3'-deoxythymidine, 6-beniylaminopurine, cytidine phosphoramidite, beta-cyanoethyl diisopropyl, 249802cytidine-5'-triphosphate, 2'-deoxyadenosine, 2'-deoxyadenosine 5'-triphosphate, 2'-deoxycytidine, 2'-deoxycytidine 5'-triphosphate, 2'-deoxyguanosine, 2'-deoxyguanosine 5'-triphosphate, 2',3'-dideoxyadenosine, 2',3'-dideoxyadenosine 5'-triphosphate, 2',3'-dideoxycytidine, 2',3'-dideoxycytidine 5'-triphosphate, 2',3'-dideoxyguanosine, 2',3'-dideoxyguanosine 5'-triphosphate, 2',3'-dideoxyinosine, 2',3' dideoxy-thymidine, 2',3'-dideoxythymidine 5'-triphosphate, 2',3'-dideoxyuridine, N6-dimethylallyladenine, 5-fluoro-2'-deoxyuridine, 5-fluorouracil, 5-fluorouridin, 5-fluorouridine 5'-monophosphate, formycine A 5'-triphosphate, formycine B, guanosine-3'-5'-cyclic monophosphate, guanosine-5'-diphosphate-3'-diphosphate, guanosine-5'-o-(2-thiotriphosphate), guanosine-5'-o-(3'-thiotriphosphate), guanosine 5'-triphosphate, 5'-guanylylimidodiphosphate, inosine, 5-iodo-2'-deoxyuridine, nicotinamide-adenine dinucleotides, nicotinamide-adenine dinucleotides, nicotinamide-adenine dinucleotide phosphate, oligodeoxythymidylic acid, (p(dT)10), oligodeoiythymidylic acid (p(dT)12-18), polyadenylic acid (poly A), polyadenylic acid-oligodeoxythymidynic acid, polycytidylic acid, poly(deoxyadenyl-deoxiythymidylic acid, polydeoxyadenylic-acid-oligodeoxythymidynic acid, polydeoxythymidylic acid, polyinosine acid-polycytidylic acid, polyuridynic acid, ribonucleic acid, tetrahydrouridine, thymidine, thymidine-3',5'-diphosphate, thymidine phosphoramidite, beta-cyanoethyl diisopropyl, 606102 thymidine 5'-triphosphate, thymine, thymine riboside, uracil, uridine, uridine-5'-diphosphoglucose, uridine 5'-triphosphate, xanthine, zeatine, transeatine riboside, etc. Further suitable polymers are: poly(DA) ss, poly(A) ss, poly(C) ss, poly(G) ss, poly(U) ss, poly(DA)-(DT) ds, complementary homopolymers, poly (D(A-T)) ds, copolymers, poly(DG).(DC) ds, complementary homopolymers, poly (d(G-C)) ds copolymers, poly (d(L-C)) ds copolymers, poly(I)-poly(C) ds, etc. An oligopeptide or a polypeptide preferably contains 3-250, frequently 4-100, and very often 4-50 amino acids which are mutually coupled via amide-bonds. Suitable amino acids are usually of the alpha- and L-type; exceptions, however, such as in dermorphine are possible.

Detailed Description Text (209):
Peptides with a particularly high biological and/or therapeutic significance, and which can also be combined with transfersomes, are, for example, N-acetyl-Ala-Ala-Ala-, N-acetyl-Ala-Ala-Ala methyl ester, N-acetyl-Ala-Ala-Ala-Ala, N-acetyl-Asp-Glu, N-acetyl-Gly-Leu, Nalpha-Acetyl-Gly-Lys methyl ester acetate, acetyl-hirudine fragments, acetyl-5-hydroxy-Trp-5-hydroxy-Trp amide, des-acetyl-alpha-melanocyte stimulating hormone, N-Acetyl-Met-Asp-Arg-Val-Leu-Ser-Arg-Tyr, N-acetyl-Met-Leu-Phe, acetyl-muramyl-Ala-isoGln, N-acetyl-Phe-Tyr, N-acetyl-Phe-norLeu-Arg-Phe amide, N-acetyl-renine substrate tetradecapeptide, N-acetyl-transforming growth factor, adipokinetic hormone II, adjuvant peptide, adrenal peptide E, adrenocorticotropic hormone (ACTH 1-39, Corticotropine A) and its fragments such as 1-4 (Ser-Tyr-Ser-Met), 1-10 (Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly), 1-17, 1-24 and 1-39, 11-24, 18-39, Ala-Ala, beta-Ala-Ala, Ala-Ala-Ala, Ala-Ala-Ala methyl ester, Ala-Ala-Ala-Ala, Ala-Ala-Ala-Ala-Ala, Ala-Ala-Ala-Ala-Ala-Ala, Ala-Ala-Phe, 7-amido-4-methylcoumarin, Ala-Ala-Phe p-nitroanilide, Ala-Ala-Val-Ala p-nitroanilide, Ala-Arg-Pro-Gly-Tyr-Leu-Ala-Phe-Pro-Arg-Met amide, beta-Ala-Arg-Ser-Ala-Pro-Thr-Pro-Met-Ser-Pro-Tyr, Ala-Asn, Ala-Asp, Ala-Glu, Ala-gamma-Gln-Lys-Ala-Ala, Ala-Gly, beta-Ala-Gly, Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Tyr-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe amide, Ala-Gly-Gly, Ala-Gly-Ser-Glu, Ala-His, beta-Ala-His, Ala-isoGln-Lys-Ala-Ala, Ala-Ile, Ala-Leu, beta-Ala-Leu, Ala-Leu-Ala, Ala-Leu-Ala-Leu, Ala-Leu-Gly, Ala-Lys, beta-Ala-Lys, Ala-Met, N-beta-Ala-1 -methyl-His, Ala-norVal, Ala-Phe, beta-Ala-Phe, Ala-Phe-Lys 7-amido-4-methylcoumarin, Ala-Pro, Ala-Pro-Gly, Ala-sarcosine, Ala-Ser, Ala-Ser-Thr-Thr-Thr-AsN-Tyr-Thr, Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr amide, Ala-Thr, Ala-Trp, beta-Ala-Trp, Ala-Tyr, Ala-Val, beta-Ala-Val, beta-Ala-Trp-Met-Asp-Phe amide, alytesine, amanitine, amastatine, angiotensine I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu), II II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), III and related peptides, angiotensine II antagonist, angiotensine II receptor

binding protein, angiotensine converting enzyme and its inhibitor (e.g. entipaine, bestatine, chymostatine, E-64, elastatinal, etc.) anserine, antide, aprotinine, arginine, vasopressine-Ala-Gly, Arg-Ala, Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Gly, Arg-Asp, Arg-Glu, Arg-Gly, Arg-Gly-Asp, Arg-Gly-Asp-Ser, Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Lys-Pro, Arg-Gly-Glu-Ser, Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala, Arg-His-Phe, Arg-Ile, Arg-Leu, Arg-Lys, Arg-Lys-Asp-Val-Tyr, Arg-Phe, Arg-Phe-Asp-Ser, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, Arg-Ser-Arg, Arg-Ser-Arg-His-Phe, Arg-Val, Asn-Pro-Asn-Ala-Asn-Pro-Asn-Ala, Asn-Pro-Asn-Ala-Asn-Pro-Asn-Ala-Asn-Pro-Asn-Ala, alpha-Asp-Ala, Asp-Ala-Glu-Asn-Leu-Ile-Asp-Ser-Phe-Gln-Glu-Ile-Val, Asp-Asp, alpha-Asp-Glu, alpha-Asp-Gly, beta-Asp-Gly, beta-Asp-His, Asp-Leu amide, beta-Asp-Leu, alpha-Asp-Lys, alpha-Asp-Phe amide, alpha-Asp-Phe, alpha-Asp-Phe methyl ester, beta-Asp-Phe methyl ester, alpha-Asp-Ser-Asp-Pro-Arg, Asp-Val, beta-Asp-Val, atrial natriuretic peptide, especially its fragments 1-32 and 5-28, atriopeptine I, II and III, auriculine A and B, beauvericine, beniotript, bestatine, N-benzylated peptides, big gastrine I, bombesine, (D-Phe12,Leu14) (Tyr4), (Lys3)-bombesine, (Tyr4)-bombesine, adrenal medulla docosapeptide and dodecapeptide, Bradykinine (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and related peptides, Bradykinine potentiators, brain natriuretic peptide, buccaline, bursine, S-t-butyl-Cys, caeruleine, calcitonine, calcitonine gene related peptide I and II, calmoduline binding domain, N-carboxymethyl-Phe-Leu, N-((R,S)-2-carboxy-3-phenyl-propionyl)Leu, cardioactive peptides A and B, carnosine, beta-casomorphine, CD4, cerebelline, N-chloroacetyl-Gly-Gly, chemotactic peptides such as formylated substances, cholecystokinine fragments, e.g., cholecystokinine octapeptide, coherine etc.

Detailed Description Text (213):
Oxidoreductases, such as: alcohol dehydrogenase (1.1.1.1), alcohol dehydrogenase (NADP dependent) (1.1.1.2), glycerol dehydrogenase (1.1.1.6), glycerophosphate dehydrogenase (1.1.1.8), xylulose reductase (1.1.1.10), polyol dehydrogenase (1.1.1.14), sorbitol dehydrogenase (1.1.1.14), myo-inositol dehydrogenase (1.1.1.18), uridine 5'-diphosphoglucose dehydrogenase (1.1.1.22), glyoxalate reductase (1.1.1.26), lactate dehydrogenase (1.1.1.27), lactate dehydrogenase (1.1.1.28), glycerate dehydrogenase (1.1.1.29), beta-hydroxybutyrate dehydrogenase (1.1.1.30), beta-hydroxyacyl CoA dehydrogenase (1.1.1.35), malate dehydrogenase (1.1.1.37), malate enzyme (1.1.1.40), isocitric dehydrogenase (1.1.1.42), 6-phosphogluconate dehydrogenase (1.1.1.44), glucose dehydrogenase (1.1.1.47), beta-galactose dehydrogenase (1.1.1.48), glucose-6-phosphate dehydrogenase (1.1.1.49), 3alpha-hydroxysteroid dehydrogenase (1.1.1.50), 3beta-hydroxysteroid dehydrogenase (1.1.1.51), 3alpha,2beta-hydroxysteroid dehydrogenase (1.1.1.53), 3-phosphoglycerate dehydrogenase (1.1.1.95), fucose dehydrogenase (1.1.1.122), lactate dehydrogenase (cytochrome) (1.1.2.3), glucose oxidase (1.1.3.4), cholesterol oxidase (1.1.3.6), galactose oxidase (1.1.3.9), alcohol oxidase (1.1.3.13), glycolate oxidase (1.1.3.15), choline oxidase (1.1.3.17), glycerol-3-phosphate oxidase (1.1.3.21), xanthine oxidase (1.1.3.22), alcohol dehydrogenase (1.1.99.8), fructose dehydrogenase (1.1.99.11), formaldehyde dehydrogenase (1.2.1.1), formate dehydrogenase (1.2.1.2), aldehyde dehydrogenase (1.2.1.5), glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12), gabase (1.2.1.16), pyruvate oxidase (1.2.3.3), oxalate oxidase (1.2.3.4), dihydroorotate dehydrogenase (1.3.3.1), lipoxidase (1.3.11.12), alanine dehydrogenase (1.4.1.1), glutamic dehydrogenase (1.4.1.3), glutamate dehydrogenase (NADP) (1.4.1.4), L-amino acid oxidase (1.4.3.2), D-amino acid oxidase (1.4.3.3), monoaminoxidase (1.4.3.4), diaminoxidase (1.4.3.6), dihydrofolate reductase (1.5.1.3), 5,10-methylenetetrahydrofolat dehydrogenase (1.5.1.5), saccharopine dehydrogenase NAD+ (1.5.1.7), octopine dehydrogenase (1.5.1.11), sarcosine oxidase (1.5.3.1), sarcosine dehydrogenase (1.5.99.1), glutathione reductase (1.6.4.2), ferridoxin-NADP+ reductase (1.6.7.1), NADPH-FMN oxidoreductase (1.6.99.1), cytochrome c reductase (1.6.99.3), NADH-fmn oxidoreductase (1.6.99.3), dihydropteridin reductase (1.6.99.7), uricase (1.7.3.3), diaphorase (1.8.1.4), lipoamide dehydrogenase (1.8.1.4), cytochrome oxidase (1.9.3.1), nitrate reductase (1.9.6.1), phenolase (1.10.3.1), ceruloplasmine (1.10.3.2), ascorbate oxidase (1.10.3.3), NADH peroxidase (1.11.1.1), catalase (1.11.1.6), lactoperoxidase (1.11.1.7), myeloperoxidase (1.11.1.7), peroxidase (1.11.1.7), glutathione peroxidase (1.11.1.9), chloroperoxidase (1.11.1.10), lipoxidase (1.13.1.12), protocatechuate 3,4-dioxygenase (1.13.11.3), luciferase (glow-worm) (1.13.12.7), salicylate hydroxylase (1.14.13.7), p-hydroxybenzoate hydroxylase (1.14.13.2),

luciferase (bacterial) (1.14.14.3), phenylalanine hydroxylase (1.14.16.1), dopamine-beta-hydroxylase (1.14.17.1), tyrosinase (1.14.18.1), superoxide dismutase (1.15.1.1), ferredoxine-NADP reductase (1.18.1.2), etc.. Transferases, such as: catecholic o-methyltransferase (2.1.1.6), phenylethanol-amine N-methyl-transferase (2.1.1.28), aspartate transcarbamylase (2.1.3.2), ornithine carbamyltransferase (2.1.3.3), transketolase (2.2.1.1), transaldolase (2.2.1.2), choline acetyltransferase (2.3.1.6), carnitine acetyltransferase (2.3.1.7), phosphotransacetylase (2.3.1.8), chloroamphenicol acetyltranferase (2.3.1.28), kanamycine 6'-acetyltransferase (2.3.1.55), gentamicine acetyltransferase (2.3.1.60), transglutaminase (2.3.2.13), gamma-glutamyl transpeptidase (2.3.2.2), phosphorylase A (2.4.1.1), phosphorylase B (2.4.1.1), dextransucrase (2.4.1.5), sucrose phosphornase (2.4.1.7), glycogen synthase (2.4.1.11), uridine 6'-diphosphoglucuronyltransferase (2.4.1.17), galactosyl transferase (2.4.1.22), nucleoside phosphorylase (2.4.2.1), orotidine-5'-monophosphate pyrophosphorylase (2.4.2.10), glutathione s-transferase (2.5.1.18), glutamine-oxalate transaminase (2.6.1.1), glutamic-pyruvate transaminase (2.6.1.2), gabase (2.6.1.19), hexokinase (2.7.1.1), galactokinase (2.7.1.6), fructose-9-phosphate kinase (2.7.1.11), gluconate kinase (2.7.1.12), phosphoribulokinase (2.7.1.19), NAD kinase (nicotinamide adenine dinucleotide kinase) (2.7.1.23), glycerokinase (2.7.1.30), choline kinase (2.7.1.32), protein kinase (3':5'-cyclic-AMP dependent) (2.7.1.37), phosphorylase kinase (2.7.1.38), pyruvate kinase (2.7.1.40), fructose-9-phosphate kinase (pyrophosphate dependent) (2.7.1.50), acetate kinase (2.7.2.1), carbamate kinase (2.7.2.2), 3-phosphoglyceric phosphokinase (2.7.2.3), creatine phosphokinase (2.7.3.2), etc.

Detailed Description Text (214):
Transpeptidases, such as: esterase (3.1.1.1), lipase (3.1.1.3), phospholipase A (3.1.1.4), acetylesterase (3.1.1.6), cholinesterase, acetyl (3.1.1.7), cholineesterase, butyryl (3.1.1.8), pectinesterase (3.1.1.11), cholesterol esterase (3.1.1.13), glyoxalase ii (3.1.2.6), phosphatase, alkaline (3.1.3.1), phosphatase acid (3.1.3.2), 5'-nucleotidase (3.1.3.5), 3'-nucleotidase (3.1.3.6), glucose-6-phosphatase (3.1.3.9), fructose-1,6-diphosphatase (3.1.3.11), phytase (3.1.3.26), phosphodiesterase i (3.1.4.1), glycerophosphorylcholine (3.1.4.2), phospholipase C (3.1.4.3), phospholipase D (3.1.4.4), deoxyribonuclease I (3.1.4.5), deoxyribonuclease II (3.1.4.6), ribonuclease N1 (3.1.4.8), sphingomyelinase (3.1.4.12), phosphodiesterase 3':5'-cyclic (3.1.4.17), phosphodiesterase II (3.1.4.18), endonuclease (3.1.4.21), ribonuclease A (3.1.4.22), ribonuclease B (3.1.4.22), 3'-phosphodiesterase 2':3'-cyclic nucleotide (3.1.4.37), sulfatase (3.1.6.1), chondro-4-sulfatase (3.1.6.9), chondro-6-sulfatase (3.1.6.10), ribonuclease T2 (3.1.27.1), ribonuclease Ti (3.1.27.3), ribonuclease u2 (3.1.27.4), nuclease (3.1.30.1), nuclease, (from micrococces) (3.1.31.1), alpha-amylase (3.2.1.1), beta-amylase (3.2.1.2), amyloglucosidase (3.2.1.3), cellulase (3.2.1.4), laminarinase (3.2.1.6), dextranase (3.2.1.11), chitinase (3.2.1.14), pectinase (3.2.1.15), lysozyme (3.2.1.17), neuraminidase (3.2.1.18), alpha-glucosidase, maltase (3.2.1.20), beta-glucosidase (3.2.1.21), alpha-galactosidase (3.2.1.22), beta-galactosidase (3.2.1.23), alpha-mannosidase (3.2.1.24), beta-mannosidase (3.2.1.25), invertase (3.2.1.26), trehalase (3.2.1.28), beta-N-acetylglucosaminidase (3.2.1.30), beta-glucuronidase (3.2.1.31), hyaluronidase (3.2.1.35), betaxylosidase (3.2.1.37), hesperidinase (3.2.1.40), pullulanase (3.2.1.41), alpha-fucosidase (3.2.1.51), mycodextranase (3.2.1.61), agarase (3.2.1.81), endoglycosidase F (3.2.1.96), endo-alpha-N-acetylgalactosaminidase (3.2.1.97), NADase (nicotinamide adenine glycopeptidase) F (3.2.2.5), dinucleotidase (3.2.2.18), thiogluc (3.2.3.1), s-adenosylhomocystein-hydrolase (3.3.1.1), leucin-aminopeptidase, (from cytosol) (3.4.11.1), leucin-aminopeptidase, microsomale (3.4.11.2), pyroglutamateaminopeptidase (3.4.11.8), carboxypeptidase a (3.4.12.2), carboxypeptidase B (3.4.12.3), prolidase (3.4.13.9), cathepsin C (3.4.14.1), carboxypeptidase W (3.4.16.1), carboxypeptidase A (3.4.17.1), carboxypeptidase B (3.4.17.2), alpha-chymotrypsin (3.4.21.1), betachymotrypsin (3.4.21.1), gamma-chymotrypsin (3.4.21.1), delta-chymotrypsin (3.4.21.1), trypsin (3.4.21.4), thrombin (3.4.21.5), plasmin (3.4.21.7), kallikrein (3.4.21.8), enterokinase (3.4.21.9), elastase from pancreas (3.4.21.11), protease (subtilisin) (3.4.21.14), urokinase (3.4.21.31), elastase from leucocytes (3.4.21.37), cathepsin B, (3.4.22.1), papain (3.4.22.2), ficin (3.4.22.3), bromo-elain (3.4.22.4), chymopapain (3.4.22.6), clostripain (3.4.22.8), proteinase A (3.4.22.9), pepsine (3.4.23.1), renine (3.4.23.4), cathepsin D (3.4.23.5), protease (aspergillopeptidase)

(3.4.23.6), collagenase (3.4.24.3), collagenase (3.4.24.8), pinguinain (3.4.99.18), renine (3.4.99.19), urokinase (3.4.99.26), asparaginase (3.5.1.1), glutaminase (3.5.1.2), urease (3.5.1.5), acylase i (3.5.1.14), cholylglycine hydrolase (3.5.1.24), urease(ATP-hydrolyzing) (3.5.1.45), penicillinase (3.5.2.6), cephalosporinase (3.5.2.8), creatininase (3.5.2.10), arginase (3.5.3.1), creatinase (3.5.3.3), guanase (3.5.4.3), adenosine-deaminase (3.5.4.4), 5'-adenylate acid-deaminase (3.5.4.6), creatinine deiminase (3.5.4.21), anorganic pyrophosphatase (3.6.1.1), adenosine 5'-triphosphatase (3.6.1.3), apyrase (3.6.1.5), pyrophosphatase, nucleotide (3.6.1.9), etc.

Detailed Description Text (215):
Lyases, such as: pyruvate-decarboxylase (4.1.1.1), oxalate decarboxylase (4.1.1.2), oxalacetate decarboxylase (4.1.1.3), glutamic decarboxylase (4.1.1.15), ornithine decarboxylase (4.1.1.17), lysine decarboxylase (4.1.1.18), arginin decarboxylase (4.1.1.19), histidine decarboxylase (4.1.1.22), orotidine 5'-monophosphate decarboxylase (4.1.1.23), tyrosine decarboxylase (4.1.1.25), phospho(enol) pyruvate carboxylase (4.1.1.31), ribulose-1,5-diphosphate carboxylase (4.1.1.39), phenylalanine decarboxylase (4.1.1.53), hydroxymandelonitrilelyase (4.1.2.11), aldolase (4.1.2.13), N-acetylneuramine acid aldolase (4.1.3.3), etc. citrate lyase (4.1.3.6), citrate synthase (4.1.3.7), tryptophanase (4.1.99.1), isozymes of carbonic anhydrase (4.2.1.1), fumarase (4.2.1.2), aconitase (4.2.1.3), enolase (4.2.1.11), crotonase (4.2.1.17), delta-aminolevulinate dehydratase (4.2.1.24), chondroitinase ABC (4.2.2.4), chondroitinase AC (4.2.2.5), pectolyase (4.2.2.10), aspartase (4.3.1.1), histidase (4.3.1.3), phenylalanine ammonia-lyase (4.3.1.5), argininosuccinate lyase (4.3.2.1), adenylosuccinate lyase (4.3.2.2), glyoxalase II (4.4.1.5), isomerases, such as: ribulose-5'-phosphate 3-epimerase (5.1.3.1), uridine 5'-diphosphogalactose 4-epimerase (5.1.3.2), mutarotase (5.1.3.3), triosephosphate isomerase (5.3.1.1), phosphoriboisomerase (5.3.1.6), phosphomannose isomerase (5.3.1.8), phosphoglucose isomerase (5.3.1.9), tautomerase (5.3.2.1), phosphoglucomutase (5.4.2.2), ligases, e.g.: aminoacyl-tRNA synthetase (6.1.1), s-acetyl coenzyme A synthetase (6.2.1.1), succinic thiokinase (6.2.1.4), glutamine synthetase (6.3.1.2), pyruvate carboxylase (6.4.1.1), etc.

Detailed Description Text (216):
The following are, amongst others, referred to as proteases: aminopeptidase M, amino acid-arylamidase, bromo-elaine, carboxypeptidase A, carboxypeptidase B, carboxypeptidase P, carboxypeptidase Y, cathepsine C, chymotrypsine, collagenases, collagenase/dispase, dispase, elastase, endoproteinase Arg-c, endoproteinase Asp-n sequencing grade, encloproteinase Glu-c (proteinase V8), endoproteinase Glu-c sequencing grade, endoproteinase Lys-c, endoproteinase Lys-c sequencing grade, endoproteinases, factor Xa, ficine, kallikrein, leucine-aminopeptidase, papaine, pepsine, plasmin, pronase, proteinase K, proteinase V8 (endoproteinase Glu-c), pyroglutamate-aminopeptidase, pyroglutamate-aminopeptidase, restriction protease factor Xa, subtilisine, thermolysine, thrombine, trypsine, etc.

Detailed Description Text (218):
Another class of proteins, which are important in the context of this invention, are lectins. Plants, and sometimes also animal, tissues are suitable sources of lectins; particularly convenient sources are Abrus pregatorius, Agarigus bisporus, Agrostemma githago, Anguilla anguilla, Arachis hypogaea, Artogarpus integrifolia, Bandeiraea simplicifolia BS-I und BS-II, (Griffonia simplicifolia), Banhlula purpurea, Caragana arborescens, Cicer arietinum, Canavalia ensiformis (jack bean), Caragana arborescens (Siberian pea tree), Codium fragile (green algae), Concanavalin A (Con A), Cytisus scoparius, Datura stramonium, Dolichos biflorus, Erythrina corallodendron, Euonymus europaeus, Gelonium multiflorum, Glycine max (soy), Griffonia simplicifolia, Helix aspersa (garden snail), Helix pomatia (escargot), Laburnum alpinum, Lathyrus odoratus, Lens culinaris (lentil), Limulus polyphemus, Lycopersicon esculentum (tomato), Lotus tetragonolobus, Luffa aegyptiaca, Maclura pomifera (Osaga orange), Momordica charantia (bitter pear melon), Naja mocambique (Mozambiquan cobra), Naja Naja kaouthia, Mycoplasma gallisepticum, Perseau americana (avocado), Phaseolus coccineus (beans), Phaseolus limensis, Phaseolus lunatus, Phaseolus vulgaris, Phytolacga americana, Pseudomonas aeruginosa PA-I, Pisum sativum (pea), Ptilota plumosa (red algae), Psophocarpus tetragonolobus (winged bean), Ricinus communis (castor bean), Robinia pseudoacacia (false acacia, black locust), Sambucus nigra (clematis), Saponaria officinalis, Solanum tuberosum (potato), Sophora japonica,

Tetragonolobus purpureas (winged or asparagus pea), (Lotus tetragono lobus), Tritigum vulgaris (wheat germ), Ulex europaeus, Vicia faba, Vicia sativa, Vicia villosa, Vigna radiata, Viscum album (mistle), Wisteria floribunda, etc.

Detailed Description Text (294):
Increasing volumes of Tween 80 are pipetted into appropriate volumes of an alcoholic PC solution. This gives rise to a concentration series with 12.5 through 25 mol-% surfactant (L/S=4-8). In addition to this, samples with L/S=2 and 3 are also made. After the addition of buffer, lipid vesicles are formed spontaneously: prior to further use, these are made somewhat smaller, with the aid of a 0.8 micrometer filter.

Detailed Description Text (301):
First Tween 80 and subsequently phosphate buffer are added to appropriate quantities of PC. The resulting mixture is agitated at room temperature for 4 days. The further procedure is as described in examples 40-49.

Detailed Description Text (337):
A 10% PC-suspension in isotonic solution of sodium chloride is homogenized at 22.degree. C. until the mean size of lipid vesicles is approx. 400 nm. This suspension is then distributed in aliquots of approx. 4.8 ml. A sufficient volume of Triton X-100 is pipetted into each of these aliquots to give a concentration series with nominal PC/Triton ratios in the range of 0.25 through 4 in steps of 0.5. All resulting samples are occasionally mixed and incubated at 4.degree. C. for 14 days.

Detailed Description Text (339):
The optical density (OD (400 nm)) of a lipid-triton mixture after a 10-fold dilution provides insight into the vesicle solubilization; this is represented in the right panel of FIG. 8. The solubilization limit is approx. 2 triton molecules per PC-molecule. Right below this limit, the optical density (OD (400 nm))--and thus the vesicle diameters--attain the greatest values. At PC/triton ratios higher than 2,5/1, the change in the optical density of given suspensions is only minimal.

Detailed Description Text (341):
In order to evaluate the permeation capability of the resulting lipid vesicles and transfersomes all suspensions were pressed through fine-pore filters (0.22 micrometer), as described in examples 1-13. The required pressure increases gradually with the decreasing total triton concentration in the suspension; for L/S ratios higher than 2/1 this significantly limits the permeation capability of carriers.

Detailed Description Text (428):
In order to assess the biological activity of insulin, approx. 2 hours before the sample application, a permanent, soft catheter is placed into a vein in the right hand. Every 15-45 minutes, 1-1.5 ml of blood are collected from this catheter; the first 0.5-1 ml thereof are discarded; the remaining 0.5 ml are measured with a standard enzymatic glucose test. In each case three determinations with three to four independent specimens are made. The corresponding experimental data is summarized in FIG. 13. It shows that transfersomes mediate a significant hypoglycemia in the peripheral blood some 90 minutes after the drug application; this effect lasts for approx. 2 hours and amounts to approx. 50% of the magnitude of the hypoglycemic effect of a comparable dose of subcutaneously applied insulin; the effect of the former lasts 200% longer, however.

Detailed Description Text (445):
Different amounts of phospholipid and surfactant in each experiment are weighed or pipetted into 25 ml of buffer at ratios which yield suspensions with 0-32,5 mol-% of Tween 80 and a constant total lipid concentration of 2%. Specimens are sterilized by filtering, filled into sterile glass vials and aged for 4 through 34 days. Then, the optical density of each sample is determined. This depends strongly on surfactant concentration but hardly on time within the framework of measuring conditions.

Detailed Description Text (448):
This procedure is an alternative or a supplement to the permeation resistance measurements as described in examples 40-49. FIG. 16 shows, for example, that the

amount of surfactant required for good mechanical deformability in the case of Tween 80 is 2-3 times lower than the corresponding solubilization concentration. This result is in good accord with the results of the permeation experiments.

Detailed Description Text (462):
Appropriate quantities of both lipids are dissolved in corresponding amounts of ethanol and mixed with a standard solution of insulin. 12 hours later, the crude carrier suspension is homogenized by means of filtration. Average vesicle diameter is 225.+-.61 nm and nominal insulin concentration is 83 I.U. Over an area of appr. 10 square centimeters on the right forearm 0.36 ml (30 I.U.) of insulin in transfersomes are distributed. Blood samples are taken every 10 minutes through a heparinized soft catheter positioned in a vein in the right forearm; the first 0.5 ml are always discarded; the following 0.5-0.8 ml of each sample are sedimented and immediately frozen; the remainder of each sample is used for the determination of blood glucose concentration during the experiment.

Detailed Description Text (464):
These liposomes with a relatively high surfactant concentration have only a very limited capability of transporting insulin across skin, as is seen from FIG. 17. Depending on the choice of data used for evaluation, the lowering of the blood glucose level does not exceed 2 to 5 mg/dl over a period of 30-40 minutes at the most. The effect of a comparable subcutaneous injection is 50 to 200 times higher. Surfactant-containing liposomes, which have not been optimized with regard to their `transfersomal` properties, are consequently poorly suited for the use as carriers in the case of dermal applications. Surfactant concentration in such carriers thus cannot mediate an optimal agent permeation through skin.

Detailed Description Text (473):
It is advantageous if an edge active substance is a nonionic, a zwitterionic, an anionic or a cationic surfactant. It can also contain an alcohol residue; quite suitable components are long-chain fatty acids or fatty alcohols, alkyl-trimethyl-ammonium-salts, alkylsulfate-salts, cholate-, deoxycholate-, glycodeoxycholate-, taurodeoxycholate-salts, dodecyl-dimethyl-aminoxide, decanoyl- or dodecanoyl-N-methylglucamide (MEGA 10, MEGA 12), N-dodecyl-N,N-dimethylglycine, 3-(hexadecyldimethylammonio)-propanesulfonate, N-hexadecylsulfobetaine, nonaethyleneglycol-octylphenylether, nonaethylene-dodecylether, octaethyleneglycol-isotridecylether, octaethylene-dodecylether, polyethylene glycol-20-sorbitane-monolaurate (Tween 20), polyethylene glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylenecetylstearylether (Cetomacrogo, Cremophor O, Eumulgin, C 1000) polyhydroxyethylene-4-laurylether (Brij 30), polyhydroxyethylene-23-laurylether (Brij 35), polyhydroxyethylene-8-stearate (Myrj 45, Cremophor AP), polyhydroxyethylene-40-stearate (Myrj 52), polyhydroxyethylene-100-stearate (Myrj 59), polyethoxylated castor oil 40 (Cremophor EL), polyethoxylated hydrated castor oil, sorbitane-monolaurate (Arlacel 20, Span 20), especially preferred decanoyl- or dodecanoyl-N-methylglucamide, lauryl- or oleoylsulfate-salts, sodiumdeoxycholate, sodiumglycodeoxycholate, sodiumoleate, sodiumelaidate, sodiumlinoleate, sodiumlaurate, nonaethylene-dodecylether, polethylene-glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylene-23-lauryl ether (Brij 35), polyhydroxyethylene-40-stearate (Myrj 52), sorbitane-monolaurate (Arlacel 20, Span 20) etc.

Detailed Description Text (495):
At irregular intervals of between 15 and 40 minutes, blood samples are drawn from a soft i.v. catheter placed in the left forearm. The determination of the blood glucose level is performed as described in example 166.

Detailed Description Text (496):
The course in time of the transfersome mediated hypoglycemia is represented in FIG. 18. The blood glucose level decreases approx. 1.5 hours after drug application by some 10 mg/ml; this artificial hypoglycemia lasts for 4 hours at least and thus attains 70-80% of the value which can be achieved by a subcutaneous application of a comparable amount of the drug Actrapid. The results of control experiments in which the insulin containing transfersomes are injected subcutaneously are shown as crosses in this figure. The total effect in the latter case is similar to that induced by the free drug injected s.c.

Detailed Description Text (502):
Starting glucose concentration in the blood of a test person (70 kg, 37 years, normoglycemic, starved for 24 hours) is measured over a period of 90 minutes for reference. Subsequently, the above-mentioned transfersome suspension with a nominal concentration of 85 I.U. insulin/ml, which has been aged for 12 hours at 4.degree. C., is applied on the right forearm skin (approx. 330 .mu.l over an area of approx. 15 cm.sup.2); this corresponds to a total applied dose of 28 I.U.

Detailed Description Text (504):
Blood specimens are collected through a heparinized, permanent, soft catheter placed in a vein in the left forearm; 0.5 ml of each sample are sedimented and immediately frozen for further use. The remaining volume is used for the in situ determination of the blood glucose concentration by an enzymatic method. The measured glucose concentration decreases by approx. 8 mg/dl after approx. 2.5 hours and remains diminished for more than 4.4 hours. This corresponds to 75% of the maximally achievable effect, as concluded from control experiments performed by injecting insulin s.c. The pharmacokinetics of this experimental series is represented in FIG. 19.

Detailed Description Text (518):
The resulting decrease of the blood glucose level after 4 hours amounts to 7.8 mg/dl and after 6 hours to 8.5 mg/dl. It is thus comparable to the result obtained in experiment no. 238.

Detailed Description Text (530):
The results of these two experiments are given in FIG. 21. They show that preparations with a relatively high surfactant concentration (Sample 1, L/S=3/1) can cause a hardly significant decrease in the blood glucose level; transfersomes close to their optimum, however, with a surfactant concentration lower by approx. 30% (L/S=4.5/1), cause a very pronounced `hypoglycemia` which lasts for many hours.

Detailed Description Paragraph Table (5):
301.3-335.4 mg phosphatidylcholine from soy-bean (+95% = PC) 123.3-80.8 .mu.l Tween 80 (puriss.) 0.38-0.42 ml ethanol, absolute 4.5 ml phosphate buffer, isotonic, sterile

Detailed Description Paragraph Table (6):
314.2-335.4 mg soy-bean phosphatidylcholine (+95% = PC) 107.2-80.8 ml Tween 80 (puriss.) 4.5 ml phosphate buffer, isotonic, sterile

Detailed Description Paragraph Table (11):
121.2-418.3 mg phosphatidylcholine from soy-bean (Grade I, PC) 378.8-81.7 mg Triton X-100 4.5 ml 0.9% NaCl solution in water

Detailed Description Paragraph Table (26):
1.1-2 mg phosphatidylcholine from soy-bean (+95% = PC) 0-32.5 mol-% Tween 80 pH 7.2 isotonic phosphate buffer

Other Reference Publication (11):
E.C. Katoulis et al., "Efficacy of a New Needleless Insulin Delivery System Monitoring of Blood Glucose Fluctuations and Free Insulin Levels" on International Journal of Artifical Organs vol. 12, No. 5, 1989, pp 333-338. (Exhibit L).

CLAIMS:

29. The method of claim 1, wherein said surfactant is selected from the group consisting of lysophosphatidic acid, lysophosphoglycerol, deoxycholate, glycodeoxycholate, laurate, myristate, oleate, palmitoleate, phosphate salts thereof, sulfate salts thereof, a Tween-surfactant and a Myrj-surfactant.

L7: Entry 6 of 12 File: USPT Oct 24, 2000

DOCUMENT-IDENTIFIER: US 6136323 A
TITLE: Bacterial plasmin receptors as fibrinolytic agents

Brief Summary Text (7):
The biochemical interactions occurring at cell surfaces between bacterial membranes and their surroundings are complex and not well understood. Certain bacterial surface structures and secreted products have been suggested to contribute to tissue invasion. One of these secreted products, streptokinase, is a plasminogen activator and converts the host zymogen plasminogen to the active protease, plasmin (Siefring, G. E., F. J. Castellino [1976] J. Biol. Chem. 251:3913-3920). Although classically described as the enzyme responsible for fibrin degradation, plasmin is a serine protease with trypsin-like specificity and has activity for a broad range of substrates. Plasmin can degrade several mammalian extracellular matrix proteins, such as fibronectin and laminin, and can enhance collagenase activity (Liotta, L. A., R. H. Goldfarb, R. Brundage [1981] Cancer Res. 41:4629-4636). Therefore, the ability to generate and capture active plasmin may contribute to the invasive propensity of certain streptococcal strains. The interaction of plasmin with group A streptococci has high affinity (K.sub.d, 10.sup.-10 M) and is specific for plasmin, with no significant binding demonstrated for structurally related proteins (Broeseker, T. A., M. D. P. Boyle, R. Lottenberg [1988] Microb. Pathog. 5:19-27; DesJardin, L. E., M. D. P. Boyle, R. Lottenberg [1989] Thromb. Res. 55:187-193).

Brief Summary Text (8):
As described herein, the plasmin receptor of the subject invention has significant similarity to the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH). GAPDH is a key enzyme involved in glucose metabolism and has been the subject of many genetic studies. Multiple copies of GAPDH genes have been identified for mammals, with many described as pseudogenes (Piechaczyk, M., J. M. Blanchard, S. Riaad-El Sabouty, C. Dani, L. Marty, P. Jeanteur [1984] Nature 312:469-471). Multiple GAPDH genes have also been identified for E. coli, Tiypanosoma brucei, Saccharomyces cerevisiae, and Drosophila melanogaster (Alefounder, P. R., R. N. Perham [1989] Mol. Microbiol. 3:723-732; Holland, J. P., L. Banieniec, C. Swimmer, M. J. Holland [1983] J. Biol. Chem. 258:5291-5299; Michels, P. A. M., M. Marchand, L. Kohl, S. Allert, R. K. Wierenga, F. R. Opperdoes [1991] Eur. J. Biochem. 198:421-428; Tso, J. Y., X. -H. Sun, R. Wu [1985] Nucleic Acids Res. 17:1251). E. coli and T. brucei each have two GAPDH genes with significant differences in deduced amino acid sequence (Alefounder et al., supra; Michels et al., supra); however, the translated product of the second E. coli GAPDH gene has not been reported. One of the trypanosomal isoenzymes is localized in the glycosome, a specialized metabolic organelle, while the other GAPDH is found in the cytoplasm (Lambier, A. -M., A. M. Loiseau, D. A. Kuntz, F. M. Vellieux, P. A. M. Michels, F. R. Opperdoes [1991] Eur. J. Biochem. 198:429-435).

Brief Summary Text (9):
In addition to its usual intracellular location, GAPDH has been identified on the surface of hematopoietic cells and Schistosoma mansoni, an invasive parasite (Allen, R. W., K. A. Trach, J. A. Hoch [1987] J. Biol. Chem. 262:649-653; Goudot-Crozel, V., D. Caillol, M. Djabali, A. J. Dessein [1989] J. Exp. Med. 170:2065-2080). Allen and Hoover ([1985] Blood 65:1045-1055) characterized a membrane-associated 37,000-M.sub.r protein expressed by the erythroleukemic cell line K562. Peptide mapping and molecular cloning studies revealed the protein to be homologous to GAPDH (Allen, Trach, and Hoch, supra). A similar finding has been reported for the blood

fluke responsible for abdominal schistosomiasis (Goudet-Crozel et al., supra). A 37,000-M.sub.r surface immunogen of S. mansoni was characterized by isolating the cDNA encoding the protein. The deduced amino acid sequence had significant homology to that of human GAPDH. Like the recombinant plasmin receptor protein (Plr), neither of these surface proteins had domains corresponding to previously described membrane-anchoring structures (Blobel, G. [1980] Proc. Natl. Acad. Sci. USA 77:1496-1500; Ferguson, M. A. J., A. F. Williams [1988] Annu. Rev. Biochem. 57:285-320). Interestingly, Hekman et al. (Hekman, W. E., D. T. Dennis, J. A. Miemyk [1990] Mol. Microbiol. 4:1363-1369), while studying the expression of recombinant plant GAPDH in E. coli, were able to target the protein to the outer membrane by genetically fusing the signal sequence of E. coli OmpA to Ricinus communis GAPDH. Pancholi et al. have recently reported the isolation of a 39 kD surface protein with GAPDH activity from a group A streptococci (Pancholi, V., V. A. Fischetti [1992] "A Novel Multifunctional Surface Protein (MFG) of group A Streptococci," Abstract No. B-252, Abstracts of the General Meeting 1992:68).

Detailed Description Text (2):
SEQ ID NO. 1 shows the composite DNA and translated amino acid sequences of the plasmin receptor protein of the subject invention.

Detailed Description Text (3):
SEQ ID NO. 2 is the translated amino acid sequence of the plasmin receptor protein of the subject invention.

Detailed Description Text (12):
We have isolated and analyzed plr, the gene encoding a group A streptococcal plasmin receptor. By screening a .lambda.gt11 expression library with antiplasmin receptor antibodies, we identified a plasmin receptor gene within a 2.7-kb streptococcal DNA fragment. This fragment was subcloned into a low-copy-number plasmid, and the receptor protein was stably expressed in E. coli under the control of putative streptococcal promoter elements. The recombinant receptor protein demonstrated immunoreactivity and plasmin-binding activity. We determined the nucleotide sequence for plr and upstream elements of the structural gene. An open reading frame of 1,008 bp was identified. The 40.5% G+C content of plr was slightly higher than the 35 to 39% reported for group A streptococcal chromosomal DNA (Hardie, J. M. [1986] "Genus Streptococcus," pp. 1043-1071, In. J. G. Holt et al (eds.), Bergey's manual of systematic bacteriology, Williams & Wilkins, Baltimore). The deduced amino acid sequence was identical for 74 amino acid residues at the N terminus as well as three cyanogen bromide fragments obtained from the native streptococcal protein. The amino acid sequence obtained for the streptococcal receptor protein revealed that the initial methionine residue is cleaved.

Detailed Description Text (13):
The deduced amino acid sequence for the recombinant plasmin receptor protein (Plr) was compared with published sequences for other proteins. Plr exhibits significant similarity to the glycolytic enzyme GAPDH, reported for a number of prokaryotic and eukaryotic organisms. The best match was with B. subtilis (56% identical and 73% conserved amino acid residues). GAPDH from streptococci has not been isolated or characterized, and the relationship of the plasmin receptor to the glycolytically active enzyme remains to be defined. However, the extensive amino acid homology and similar hydropathy plots for Plr and B. subtilis GAPDH strongly suggest that Plr is a member of the GAPDH family of proteins. Furthermore, preliminary analysis of the recombinant protein revealed that Plr has GAPDH enzymatic activity.

Detailed Description Text (17):
In addition to the plasmin receptor amino acid sequence disclosed herein, the subject invention further comprises equivalent plasmin receptor proteins (and nucleotide sequences coding for equivalent proteins) having the same or similar biological activity of the plasmin receptor exemplified herein. These equivalent proteins may have amino acid homology with the protein disclosed and claimed herein. This amino acid homology will typically be greater than 75%, preferably be greater than 90%, and most preferably be greater than 95%. The amino acid homology will be highest in certain critical regions of the protein which account for biological activity or are involved in the determination of three-dimensional configuration which ultimately is responsible for the biological activity. In this regard, certain

amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Table 1 provides a listing of examples of amino acids belonging to each class.

Detailed Description Text (18):
In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not significantly detract from the biological activity of the protein. It has been shown that proteins of identified structure and function may be constructed by changing the amino acid sequence if such changes do not alter the protein secondary structure (Kaiser, E. T. and Kezdy, F. J. [1984] Science 223:249-255). Thus, the subject invention includes mutants of the amino acid sequence depicted herein which do not alter the protein secondary structure, or if the structure is altered, the biological activity is substantially retained.

Detailed Description Text (20):
It should be apparent to a person skilled in this art that genes coding for receptor-binding proteins can be identified and obtained through several means. The specific genes may be obtained from a culture depository as described herein. Alternatively, these genes, or portions thereof, may be constructed synthetically, for example, by use of a gene machine. Variations of these genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as Bal31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which code for active fragments may be obtained using a variety of other restriction enzymes. Proteases may be used to directly obtain active fragments of these proteins.

Detailed Description Text (24):
Radioiodination of proteins. Human plasminogen isolated from plasma by chromatography on lysine-Sepharose (Lottenberg, R., F. R. Dolly, C. S. Kitchens [1985] Am. J. Hematol. 19:181-193) and streptococcal protein G (Calbiochem, San Diego, Calif.) were labeled with .sup.125 I (Amersham Corp., Arlington Heights, Ill.) by using a mild lactoperoxidase reaction with Enzymo-beads (Bio-Rad, Richmond, Calif.) (McCoy II, E., C. C. Broder, R. Lottenberg [1991] J. Infect. Dis. 164:515-521). Plasmin was generated from radiolabeled plasminogen as previously described (Broder, C. C., R. Lottenberg, G. O. von Mering, K. H. Johnston, M. D. P. Boyle [1991] J. Biol. Chem. 266:4922-4928).

Detailed Description Text (27):
Screening of the streptococcal library. The resulting non-amplified .lambda.gt11 library was diluted in 10 mM Tris-2.5 mM MgSO.sub.4 -0.01% gelatin-0.1 M NaCl, pH 7.5, and used to infect E. coli Y1090, yielding a density of 200 to 400 plaques per plate. The infected cells were mixed with 0.45% soft agar, plated on 1.2% L agar supplemented with ampicillin (50 .mu.g/ml), incubated at 42 C for 3 to 4 hours to induce lysis, and overlaid with nitrocellulose filters impregnated with 10 mM isopropylthiogalactoside (IPTG) to induce the lac promoter. After incubation at 37 C for approximately 16 hours, the filters were removed, washed, and blocked in 100 mM Tris-300 mM NaCl-5 mM EDTA-0.05% Triton X-100-0.25% gelatin, pH 7.4 (NET-gel). The filters were then incubated with murine antiplasmin receptor antibody (Broder et al. [1991], supra) for approximately 18 hours at room temperature, washed, and then incubated with goat anti-mouse immunoglobulin G (Cappel, Organon Teknika) for 3 to 4 hours. Antigen-antibody complexes on washed filters were detected with .sup.125 I-streptococcal protein G. Autoradiographs were generated by exposing the washed nitrocellulose filters to Kodak XAR-5 film with intensifying screens at -70 C and then using automated film developing. immunoreactive plaques were isolated and purified through two additional screenings.

Detailed Description Text (32):
Amino acid sequencing. Mutanolysin-extracted proteins (Broder et al. [1991], supra) from strain 64/14 were subjected to SDS-PAGE. The proteins were electrotransferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore Corp., Bedford, Mass.) by using 10 mM 2-(N-morpholino)ethanesulfonic acid (pH 6.0)-20% methanol as the transfer buffer. Protein bands were stained with Coomassie brilliant blue. The .apprxeq.41,000-M.sub.r protein band, which had previously been shown to bind plasmin, was excised. Microsequencing by automated Edman chemistry was performed with an Applied Biosystems model 470A gas-phase sequencer with an on-line 12A PTH analyzer (Washington University Protein Chemistry Laboratory). Cyanogen bromide fragmentation of the .apprxeq.41,000-M.sub.r protein was performed by immersing the polyvinylidene difluoride membrane-bound protein in 70% formic acid and treating with cyanogen bromide overnight at room temperature. The fragments were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane as described above. Four peptides were identified and sequenced at the University of Florida Interdisciplinary Center for Biotechnology Research Protein Chemistry Core Facility by using an Applied Biosystems model 470 sequencer with an on-line PTH analyzer.

Detailed Description Text (36):
Plasminogen was prepared from human plasma by chromatography on lysine sepharose (Sigma Chem. Co., St. Louis, Mo., U.S.A.) and molecular sieving chromatography on Sephadex G-100 (Lottenberg, R., F. R. Dolly, C. S. Kitchens [1985] Am. J. Hematol. 19:181-193). The purified protein appeared as a single band on a silver stain of an SDS-polyacrylamide gel electrophoresis. A given concentration of isolated plasminogen following activation with streptokinase demonstrated the predicted theoretical amidolytic activity, thereby confirming the purity of the isolated human plasminogen.

Detailed Description Text (42):
Plasmin was generated from radiolabeled plasminogen by incubation with urokinase (20 units/ml, Sigma Chemical Co., St. Louis, Mo., U.S.A.) in VBS-gel that contained 0.02 M lysine. Conversion was maximal after 30 min at 37 C. The conversion of the single chain zymogen molecule to heavy and light chains was monitored, following reduction, on SDS-PAGE using the method of Laemmhi as described previously (Lottenberg, R., C. C. Broder, M. D. P. Boyle [1987] Infect. Immmun. 55:1914-1918). Greater than 95% of plasminogen was consistently converted to plasmin. The specific activity of labeled plasmin was therefore essentially the same as labeled plasminogen.

Detailed Description Text (45):
The group A .beta.-hemolytic streptococcal strain 64 had been previously subjected to mouse passage (Reis, K. J., M. Yarnall, E. M. Ayoub, M. D. P. Boyle [1984] Scand. J. Immunol. 20:433-439). The parent strain (64/P), as well as strains isolated after three (64/3) and fourteen (64/14) passages, were grown in Todd-Hewitt broth (DIFCO, Detroit, Mich.) overnight in phosphate-buffered saline (PBS), pH 7.4, containing 0.05% Tween-20 and 0.02% sodium azide. The bacteria were heat killed at 80 C for 5 min, a treatment that did not alter their plasmin binding potential, but eliminated the production of soluble plasminogen activators which would interfere with these studies. The suspension was centrifuged, the pellet washed twice with PBS 0.02% sodium azide. Samples were stored at -20 C. The concentration of a bacterial suspension was determined by counting bacterial chains in a Neubauer hemacytometer (Fisher Scientific, Orlando, Fla., U.S.A.)

Detailed Description Text (48):
To assess the effect of pH on the bacterium: plasmin (ogen) interaction, 50 .mu.l of labeled plasminogen of plasmin (approximately 2.times.10.sup.4 cpm) were added to 1.0 ml of VBS containing 0.05% Tween-20 adjusted to the appropriate pH. After 15 min at room temperature, 50 .mu.l of VBS containing approximately 10.sup.7 bacteria (strain 64/14) were added and the mixture was incubated at 37 C for 15 min. The bacterial suspensions were centrifuged at 1000 g for 7 min to separate bacteria from unbound labeled proteins and the pellets were washed twice with 2 ml of VBS at the appropriate pH. The radioactivity associated with the bacterial pellet in duplicate experiments was measured using a Beckman 5500 autogamma counter.

Detailed Description Text (50):
To assess the effect of ionic strength on the bacterium:plasmin (ogen) interaction,

similar studies were carried out in solutions containing different concentrations of NaCl with 0.05% Tween-20. The bacterial pellets were washed in the appropriate NaCl concentration to remove unbound labeled proteins.

Detailed Description Text (57):
Inhibition of Binding by Amino Acids

Detailed Description Text (58):
Labeled plasmin (100 .mu.l containing approximately 2.times.10.sup.4 cpm) was added to 200 .mu.l VBS-gel containing varying concentrations of epsilon-aminocaproic acid (EACA), lysine, or arginine, and incubated at 37 C for 15 min. The pH of each solution was 7.0. One hundred .mu.l of VBS-gel containing 10.sup.7 bacteria (strain 64/14) were then added and the mixture was incubated at 37 C for 15 min. The bacterial suspensions were centrifuged at 1000 g for 7 min and washed twice with 2 ml of VBS-gel containing the same concentration of amino acid present during the incubation period. The percent inhibition of binding was calculated for duplicate experiments by comparison with binding in VBS-gel alone.

Detailed Description Text (59):
The ability of EACA, lysine, or arginine to dissociate bound plasmin from the bacteria was examined in the following manner. Labeled plasmin was incubated with 10.sup.7 bacteria in VBS-gel containing no amino acid at 37 C for 15 min. The bacteria were pelleted by centrifugation and washed twice with 2 ml on VBS-gel. After determining the radioactivity associated with the bacteria, the pellets were resuspended in solutions of VBS-gel containing varying concentrations of amino acid (pH 7.0) as described above. The mixtures were incubated at 37 C for 15 min and washed twice with VBS-gel containing the appropriate amino acid concentration. The radioactivity associated with the bacteria in duplicate experiments was again measured and the percentage dissociated was calculated.

Detailed Description Text (60):
Binding of plasmin to the group A streptococcus 64/14 was inhibited by each amino acid in a concentration dependent fashion. Fifty percent inhibition of binding of plasmin to the bacteria was observed at an EACA concentration of 0.15 mM a lysine concentration of 2.0 mM, and an arginine concentration of 25 mM. In similar studies, plasmin was prebound to the group A streptococcus and a concentration dependent elution of bound radiolabel was observed on incubation with EACA, lysine, or arginine. The concentration of amino acid required to elute 50% of the bound plasmin was approximately equivalent to that required to inhibit plasmin binding by 50%.

Detailed Description Text (65):
Plasmin which had been bound to and eluted from strain 64/14 by treatment with lysine was also examined in similar binding studies. Eluted plasmin was obtained by incubating 2 ml of stock 10% wet weight/volume bacterial suspension (strain 64/14) with approximately 20 .mu.g of labeled plasmin at room temperature for 45 min. This suspension was centrifuged at 1000 g for 10 min and washed once with 10 ml of VBS-gel, and the radioactivity associated with the bacterial pellet was measured. The pellet was then resuspended in VBS-gel containing 20 mM lysine and incubated at room temperature for 30 min. The suspension was centrifuged and the supernatant recovered. Approximately 90% of the radioactivity originally associated with the bacterial pellet was dissociated by the lysine treatment. The dissociated plasmin in the supernatant was the subjected to gel filtration of a G-25 column to separate lysine from plasmin. Fractions containing plasmin were collected and stored at -20 C.

Detailed Description Text (69):
Bacterial extracts, chromatography fractions or standards were loaded into the wells of a dot-blotting manifold in 50-200 .mu.l aliquots. Commercially available group C streptokinase was used as a positive plasmin binding control in each assay. All wells were washed twice with 200 .mu.l aliquots of PBS-azide and vacuum drained. All samples were assayed in duplicate. Dot blots were blocked in 5.0 mM sodium diethylbarbiturate, 0.14 M NaCl, 0.5% gelatin, 0.15% Tween 20, 0.004% NaN.sub.3 pH 7.3. The blots were probed for 3-4 hours at room temperature in the blocking buffer containing 2.0 mM PMSF and .sup.125 I-labeled human plasmin at 3.times.10.sup.4 cpm/ml. The probed blots were then washed in 0.01 M EDTA pH 7.3, containing 0.5 M

NaCl 0.25% gelatin, 0.15% Tween 20, and 0.004% NaN.sub.3. Autoradiographs were generated by exposing the nitrocellulose blots to Kodak XAR-5 film with an intensifying screen for 15-24 hours at -70 C followed by automated film developing.

Detailed Description Text (72):
Gels intended for Western blotting were equilibrated in 25 mM Tris, 0.2 M glycine pH 8.0 containing 20% v/v methanol (electroblot buffer) for 25 minutes. Protein blotting, from SDS-PAGE gels, was performed using the `Trans-Blot SD Semi-Dry` electrophoretic transfer cell (Bio Rad, Richmond, Calif.). Blots were blocked as described for the dot-blot procedure, and probed for 3-4 hours at room temperature with radiolabeled human plasmin in either the presence or absence of 1.0 mM EACA, to identify functionally active protein bands. In studies of antigenic properties of these proteins, blots were probed with rabbit anti-plasmin receptor antibody or anti-group C streptokinase antibody by incubation with 4.3 mg IgG per ml of probing solution (approximately a 1:3000 dilution of antisera) for three hours and probed with .sup.125 I-streptococcal protein G containing 3.times.10.sup.4 cpm/ml. For probing with mouse monoclonal antibodies specific for epitopes on group C streptokinase, blots were probed with a 1:100 dilution of the monoclonal antibody stock solution for three hours, followed by probing with goat antibody specific for mouse IgG at 1.0 .mu.g/ml, followed by probing with .sup.125 I-streptococcal protein G containing 3.times.10.sup.4 cpm/ml. Blots were then washed with 0.01 M EDTA pH 7.3, containing 1.0 M NaCl, 0.25% gelatin, 0.15% Tween 20. Autoradiographs were generated by exposing the nitrocellulose blots to Kodak XAR-5 film with an intensifying screen for 15-24 hours at -70 C followed by automated film developing.

Detailed Description Text (76):
This procedure is a modification of the method described by Yarnall, M. and M. D. P. Boyle (1986) "Isolation and partial characterization of a type II Fc receptor from a group A streptococcus," Mol. Cell. Biochem. 70:57-66. Approximately 1.0 g wet weight of bacteria was suspended in 5.0 ml of 20 mM KH.sub.2 PO.sub.4, 1.0 mM EDTA, 0.02% NaN.sub.3 pH 7.0 containing 2.0 mM PMSF, 10 .mu.g/ml DNAse I and 50 .mu.g/ml mutanolysin. The suspension was vortexed and placed at 37 C for 4 hours with periodic mixing. Supernatants were collected following centrifugation to remove bacteria and debris. For these studies a commercial preparation of mutanolysin was further purified according to the method described by Siegal et al. (Siegal, J. L., S. F. Hurst, E. S. Liberman, S. E. Coleman, A. S. Bleiweis [1981]
"Mutanolysin-induced spheroplasts of Streptococcus mutans are true protoplasts," Infect. Immun. 31:303-815) to remove contaminating protease.

Detailed Description Text (79):
Human plasminogen at a concentration of approximately 5.6.times.10.sup.-5 M was activated to plasmin by incubating the sample in the presence of an approximately 60 fold lower molar concentration of urokinase. The reaction was carried out with constant agitation for one hour at 37 C in a reaction volume of 10 ml of 0.05 M Tris, 0.15 M NaCl pH 7.4 containing 40 mM lysine. A 50 .mu.l aliquot was removed and analyzed by SDS-PAGE under reduced conditions to determine the extent of conversion of the single chain plasminogen molecule to the two chain plasmin form. The remainder of the reaction mixture was flash frozen, and stored at -70 C. Preparations in which complete conversion of plasminogen to plasmin was observed were then reacted with constant rotation with a 5 fold molar excess of D-valyl-L-phenylalanyl-L-lysine chloromethyl ketone at ambient temperature with constant rotation. The enzymatically inactive plasmin was then concentrated by ammonium sulfate precipitation (4.0 g/10 ml), dialyzed at 4 C against 0.1 M MOPS buffer, pH 7.3, containing 0.02% sodium azide. The dialyzed inactive plasmin was chromatographed on a Superose 6 column (Pharmacia, Piscataway, N.J.) in 0.1 M MOPS buffer, pH 7.3.

Detailed Description Text (84):
The Affi-Prep 10-Plasmin matrix was placed in an HR 10/10 column attached to a Pharmacia FPLC chromatography system. The column was equilibrated at room temperature in 0.05 M Na.sub.2 HPO.sub.4, 0.15 M NaCl, 1.0 mM benzamidine HCl, 0.02% sodium azide pH 7.2 (equilibration buffer). Approximately 1 or 2 ml of supernatant from the mutanolysin extraction of bacterial strain 64/14 was loaded onto the column. The column was then washed with the equilibration buffer until the OD.sub.280 returned to base line. The column was then eluted with a 50 ml linear

gradient of 0.0 M-0.1 M L-Lysine in equilibration buffer, or eluted in a single step using equilibration buffer containing 0.1 M L-Lysine. The absorbance at 280 nm was continuously monitored an 1.0 ml fractions were collected. After each affinity purification procedure the column was washed with 20 ml of 2.0 M NaCl, followed by 200 ml of equilibration buffer and stored at 4 C.

Detailed Description Text (91):
A variety of conditions for solubilizing plasmin receptor activity from the group A streptococcal strain 64/14 were compared. These included hot acid, alkaline, and neutral pH extractions, extraction with the detergents TRITON X-100 with osmotic shock, acetone and TRITON X-100 extraction, and extractions with the enzymes, lysozyme, trypsin, or mutanolysin. The highest yield of soluble plasmin binding activity was found in mutanolysin extracts.

Detailed Description Text (99):
The mutanolysin extracted plasmin binding activity was subjected to further purification by affinity chromatography using an enzymatically inactivated plasmin affinity matrix prepared as described above. One ml of the mutanolysin extract of strain 64/14 was applied to the plasmin affinity column matrix in 0.05 M Na.sub.2 HFPO.sub.4, 0.15 M NaCl, 1.0 mM benzamidine HCl, and 0.02% NaN.sub.3, pH 7.2. Bound plasmin receptor activity was eluted using a 50 ml linear gradient of 0.0-0.1 M L-Lysine in 0.05 M Na.sub.2 HPO.sub.4, 0.15 M NaCl, 1.0 mM benzamidine HCl, and 0.02% NaN.sub.3, pH 7.2. The absorbance at 280 nm was monitored continuously and 1.0 ml fractions were collected. Fractions eluted from the affinity column were assayed for functional activity using a dot blotting procedure and .sup.125 I-labeled plasmin as the probe. The functional plasmin binding activity was found to bind to the immobilized plasmin matrix and could be eluted selectively with lysine. The recovered functional activity from the column corresponded to the eluted protein peak as detected by measuring absorbance at 280 nm. Identical results were obtained when a single concentration of 0.1 M L-lysine was used to elute the bound plasmin receptor activity from the plasmin affinity column.

Detailed Description Text (114):
N-terminal amino acid sequencing of the .apprxeq.41,000-M.sub.r plasmin receptor protein from strain 64/14 was performed, and an unambiguous sequence was obtained for 51 residues. Amino acid sequences were obtained

Detailed Description Text (115):
from four peptides (M.sub.r s of 3,000 to 16,000) generated by cyanogen bromide treatment of the .apprxeq.41,000-M.sub.r protein. The sequence of one of these peptides had identity with the N-terminal sequence and allowed assignment of an additional 23 residues. The deduced amino acid sequence of the open reading frame exhibited complete identity with 74 amino acid residues of the native protein, indicating that valine following the ATG initiation codon represents the N terminus of the receptor protein. The sequences of two additional peptides (13 and 27 residues) were also determined and found to correspond to residues 160 to 173 and 186 to 216, respectively. Thus, 114 of the predicted 335 amino acid residues encoded by plr have been confirmed by amino acid sequencing of the native streptococcal protein.

Detailed Description Text (119):
The deduced amino acid sequence of Plr was compared with deduced amino acid sequences for genes entered in the EMBL (release 26.0) and GenBank (release 67.0) data bases by using the TFASTA program based on the algorithm of Lipman and Pearson (Lipman, D., W. R. Pearson [1985] Science 227:1435-1441). Glyceraldehyde 3-phosphate dehydrogenases (GAPDHs) of bacterial origins (Branlant, G., C. Branlant [1985] Eur. J. Biochem. 150:61-66; Schlaepfer, B. S., W. Portmann, C. Branlant, G. Branlant, H. Zuber [1990] Nucleic Acids Res. 18:6422; Viaene, A., P. Dhaese [1989] Nucleic Acids Res. 17:1251) exhibited the greatest homology with Plr. The gram-positive Bacillus subtilis GAPDH demonstrated the highest score. The sequences showed 56% identity and 73% similarity.

Detailed Description Text (120):
Hydropathy plots of Plr and B. subtilis GAPDH were determined as described by Kyte and Doolittle (Kyte, J., R. F. Doolittle [1982] J. Mol. Biol. 157:105-132). Plr and

B. subtilis GAPDH showed similar patterns overall except for differences in the C-terminal portion of the molecules. Common cell wall-spanning and membrane-anchoring motifs have been identified for several gram-positive surface proteins (Fischetti, V. A., V. Pancholi, O. Schneewind [1991] "Common characteristics of the surface proteins from gram-positive cocci, pp. 290-294, In G. M. Dunny et al. (eds.), Genetics and molecular biologic of streptococci, lactococci, and enterococci, American Society for Microbiology, Washington, D.C.). However, no similar regions were identified for Plr. No significant amino acid sequence homology between Plr and streptokinase, the other well-characterized plasmin(ogen)-binding protein, was identified, supporting our previous biochemical and immunological analyses (Broder et al. [1991], supra).

Detailed Description Text (129):
An additional aspect of controlling potential hemorrhage accompanying administration of this agent may be the intravenous administration of epsilon-aminocaproic acid (tradename AMICAR) or tranexamic acid. Typical regimens of these anti-fibrinolytic agents would provide plasma levels of the lysine analogs to dissociate the plasmin from the bacteria (Broeseker, T. A., M. D. P. Boyle, R. Lottenberg [1988] "Characterization of the interaction of human plasmin with its specific receptor on a group A streptococcus," Microbial Pathogenesis 5:19-27) and enable rapid inactivation of plasmin by its physiological inhibitors .alpha.2-antiplasmin and .alpha.2-macroglobulin. This will also provide an approach to prepare the patient for emergency surgical procedures (e.g., coronary artery bypass grafting or administration of alternative anti-thrombotic agents (e.g., heparin).

Detailed Description Paragraph Table (2):
TABLE 1 _____ Class of Amino Acid Examples of Amino Acids _____ Nonpolar Ala, Val, Leu, Ile, Pro, Met, Phe, Trp Uncharged Polar Gly, Ser, Thr, Cys, Tyr, Asn, Gln Acidic Asp, Glu Basic Lys, Arg, His _____

Detailed Description Paragraph Table (4):
(B) LOCATION: 115..1122 - (ix) FEATURE: (A) NAME/KEY: mat.sub.-- - #peptide (B) LOCATION: 115..1122 (C) IDENTIFICATION METHOD: - # experimental #/codon.sub.-- start= 115MATION: /function=- # "High-affinity binding of plasmin(ogen - #)" /product=- # "Streptococcal plasmin receptor" /evidence=- # EXPERIMENTAL #"plr" /gene= #1 /number= #PLR /label= - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1: - ATAATAGTTC TGTTGAAAGG TTGTTGCAGA TGACTGTAAG TAATCTTTTC AC - #AATAGGTA 60 - GGGAGCATTC CCTCTAATAA TATTCTTTTG ATTTTCATAA GGAGGAAATC AC - #TA ATG 117 # Met # 1 - GTA GTT AAA GTT GGT ATT AAC GGT TTC GGT CG - #T ATC GGA CGT CTT GCA 165 Val Val Lys Val Gly Ile Asn Gly Phe Gly Ar - #g Ile Gly Arg Leu Ala # 15 - TTC CGC CGT ATT CAA AAC ATC GAA GGT GTT GA - #A GTA ACT CGT ATC AAT 213 Phe Arg Arg Ile Gln Asn Ile Glu Gly Val Gl - #u Val Thr Arg Ile Asn # 30 - GAC CTT ACA GAT CCA AAT ATG CTT GCA CAC TT - #G TTG AAA TAC GAT ACA 261 Asp Leu Thr Asp Pro Asn Met Leu Ala His Le - #u Leu Lys Tyr Asp Thr # 45 - ACT CAA GGT CGT TTT GAT GGA ACA GTT GAA GT - #T AAA GAA GGT GGA TTT 309 Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Va - #l Lys Glu Gly Gly Phe # 65 - GAA GTA AAC GGA AAC TTC ATC AAA GTT TCT GC - #T GAA CGT GAT CCA GAA 357 Glu Val Asn Gly Asn Phe Ile Lys Val Ser Al - #a Glu Arg Asp Pro Glu # 80 - AAC ATC GAC TGG GCA ACT GAT GGG GTT GAA AT - #C GTT CTT GAA GCA ACT 405 Asn Ile Asp Trp Ala Thr Asp Gly Val Glu Il - #e Val Leu Glu Ala Thr # 95 - GGT TTC TTT GCT AAA AAA GAA GCA GCT GAA AA - #A CAC TTA CAT GCT AAC 453 Gly Phe Phe Ala Lys Lys Glu Ala Ala Glu Ly - #s His Leu His Ala Asn # 110 - GGT GCT AAA AAA GTT GTT ATC ACA GCT CCT GG - #T GGA AAC GAT GTT AAA 501 Gly Ala Lys Lys Val Val Ile Thr Ala Pro Gl - #y Gly Asn Asp Val Lys # 125 - ACA GTT GTT TTC AAC ACT AAC CAC GAC ATT CT - #T GAC GGT ACT GAA ACA 549 Thr Val Val Phe Asn Thr Asn His Asp Ile Le - #u Asp Gly Thr Glu Thr 130 1 - #35 1 - #40 1 - #45 - GTT ATC TCA GGT GCT TCA TGT ACT ACA AAC TG - #T TTA GCT CCT ATG GCT 597 Val Ile Ser Gly Ala Ser Cys Thr Thr Asn Cy - #s Leu Ala Pro Met Ala # 160 - AAA GCT CTT CAC GAT GCA TTC GGT ATT CAA AA - #A GGT CTT ATG ACT ACA 645 Lys Ala Leu His Asp Ala Phe Gly Ile Gln Ly - #s Gly Leu Met Thr Thr # 175 - ATC CAC GCT TAC ACT GGT GAC CAA ATG ATC CT - #T GAC GGA CCA CAC CGT 693 Ile His Ala Tyr Thr Gly Asp Gln Met Ile Le - #u Asp Gly Pro His Arg # 190 - GGT GGT GAC CTT CGT CGT GCA CGC GCT GGT GC - #T GCA AAC ATC GTT CCT 741 Gly Gly Asp Leu Arg Arg Ala Arg Ala Gly Al - #a Ala Asn Ile Val Pro # 205 - AAC TCA ACT GGT GCT GCT AAA GCT ATC GGT CT - #T GTT ATC CCA GAA CTT 789 Asn Ser Thr Gly Ala Ala Lys Ala Ile Gly Le - #u Val Ile Pro Glu Leu 210 2 - #15 2 - #20 2 - #25 - AAC GGT AAA CTT GAC GGT GCT GCA CAA CGT GT - #T CCT GTT CCA ACT

GGA 837 Asn Gly Lys Leu Asp Gly Ala Ala Gln Arg Va - #l Pro Val Pro Thr Gly # 240 - TCA GTA ACT GAG TTG GTT GTA ACT CTT GAC AA - #A AAC GTT TCT GTT GAC 885 Ser Val Thr Glu Leu Val Val Thr Leu Asp Ly - #s Asn Val Ser Val Asp # 255 - GAA ATC AAC TCT GCT ATG AAA GCT GCT TCA AA - #C GAT AGC TTC GGT TAC 933 Glu Ile Asn Ser Ala Met Lys Ala Ala Ser As - #n Asp Ser Phe Gly Tyr # 270 - ACT GAA GAT CCA ATC GTT TCT TCA GAT ATC GT - #A GGC GTA TCA TAC GGT 981 Thr Glu Asp Pro Ile Val Ser Ser Asp Ile Va - #l Gly Val Ser Tyr Gly # 285 - TCA TTG TTT GAC GCA ACT CAA ACT AAA GTA AT - #G GAA GTT GAC GGA TCA 1029 Ser Leu Phe Asp Ala Thr Gln Thr Lys Val Me - #t Glu Val Asp Gly Ser 290 2 - #95 3 - #00 3 - #05 - CAA TTG GTT AAA GTT GTA TCA TGG TAT GAC AA - #C GAA ATG TCT TAC ACT 1077 Gln Leu Val Lys Val Val Ser Trp Tyr Asp As - #n Glu Met Ser Tyr Thr # 320 - GCT CAA CTT GTA CGT ACT CTT GAG TAC TTC GC - #A AAA ATT GCT AAA 1122 Ala Gln Leu Val Arg Thr Leu Glu Tyr Phe Al - #a Lys Ile Ala Lys # 335 # 1125 - (2) INFORMATION FOR SEQ ID NO:2: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 336 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: protein - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2: - Met Val Val Lys Val Gly Ile Asn Gly Phe Gl - #y Arg Ile Gly Arg Leu # 15 - Ala Phe Arg Arg Ile Gln Asn Ile Glu Gly Va - #l Glu Val Thr Arg Ile # 30 - Asn Asp Leu Thr Asp Pro Asn Met Leu Ala Hi - #s Leu Leu Lys Tyr Asp # 45 - Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Gl - #u Val Lys Glu Gly Gly # 60 - Phe Glu Val Asn Gly Asn Phe Ile Lys Val Se - #r Ala Glu Arg Asp Pro # 80 - Glu Asn Ile Asp Trp Ala Thr Asp Gly Val Gl - #u Ile Val Leu Glu Ala # 95 - Thr Gly Phe Phe Ala Lys Lys Glu Ala Ala Gl - #u Lys His Leu His Ala # 110 - Asn Gly Ala Lys Lys Val Val Ile Thr Ala Pr - #o Gly Gly Asn Asp Val # 125 - Lys Thr Val Val Phe Asn Thr Asn His Asp Il - #e Leu Asp Gly Thr Glu # 140 - Thr Val Ile Ser Gly Ala Ser Cys Thr Thr As - #n Cys Leu Ala Pro Met 145 1 - #50 1 - #55 1 - #60 - Ala Lys Ala Leu His Asp Ala Phe Gly Ile Gl - #n Lys Gly Leu Met Thr # 175 - Thr Ile His Ala Tyr Thr Gly Asp Gln Met Il - #e Leu Asp Gly Pro His # 190 - Arg Gly Gly Asp Leu Arg Arg Ala Arg Ala Gl - #y Ala Ala Asn Ile Val # 205 - Pro Asn Ser Thr Gly Ala Ala Lys Ala Ile Gl - #y Leu Val Ile Pro Glu # 220 - Leu Asn Gly Lys Leu Asp Gly Ala Ala Gln Ar - #g Val Pro Val Pro Thr 225 2 - #30 2 - #35 2 - #40 - Gly Ser Val Thr Glu Leu Val Val Thr Leu As - #p Lys Asn Val Ser Val # 255 - Asp Glu Ile Asn Ser Ala Met Lys Ala Ala Se - #r Asn Asp Ser Phe Gly # 270 - Tyr Thr Glu Asp Pro Ile Val Ser Ser Asp Il - #e Val Gly Val Ser Tyr # 285 - Gly Ser Leu Phe Asp Ala Thr Gln Thr Lys Va - #l Met Glu Val Asp Gly # 300 - Ser Gln Leu Val Lys Val Val Ser Trp Tyr As - #p Asn Glu Met Ser Tyr 305 3 - #10 3 - #15 3 - #20 - Thr Ala Gln Leu Val Arg Thr Leu Glu Tyr Ph - #e Ala Lys Ile Ala Lys # 335

CLAIMS:

1. A method for raising an immune response in a mammal, said method comprising the administration to said mammal of an isolated protein comprising the amino acid sequence shown in SEQ ID NO. 2.

L7: Entry 7 of 12 File: USPT Apr 6, 1999

DOCUMENT-IDENTIFIER: US 5891664 A
TITLE: Vectors and methods for recombinant production of uPA-binding fragments of the human urokinase-type plasminogen receptor (uPAR)

Brief Summary Text (9):
Many research groups have proposed that invasive tumor cells secrete matrix-degrading proteinases. A cascade of proteases including serine proteases and thiol proteases all contribute to facilitating tumor invasion. One of the crucial cascades is the plasminogen activation system. Regulation of the proteolysis can take place at many levels including tumor cell-host cell interactions and protease inhibitors produced by the host or by the tumor cells themselves. Expression of matrix-degrading enzymes is not tumor cell specific. The actively invading tumor cells may merely respond to different regulatory signals compared to their non-invasive counterparts (Liotta, 1986).

Brief Summary Text (15):
The cellular receptor for u-PA (u-PAR) was originally identified in blood monocytes and in the monocyte-like U937 cell line (Vassalli et al., 1985), and its presence has been demonstrated on a variety of cultured cells, including several types of malignant cells (Stoppelli et al., 1985, Vassalli et al., 1985, Plow et al., 1986, Boyd et al., 1988a, Nielsen et al., 1988), human fibroblasts (Bajpai and Baker, 1985), and also in human breast carcinoma tissue (Needham et al., 1987). The receptor binds active 54 kD u-PA, its one-polypeptide chain proenzyme, pro-u-PA (see below), as well as 54 kD u-PA inhibited by the active site reagent DFP, but shows no binding of the low molecular weight (33 kD) form of active u-PA (Vassalli et al., 1985; Cubellis et al., 1986). Thus, binding to the receptor does not require the catalytic site of u-PA, and in agreement with these findings, the binding determinant of u-PA has been identified in the amino-terminal part of the enzyme, in a region which in the primary structure is remote from the catalytic site. The receptor binding domain is located in the 15 kD amino-terminal fragment (ATF, residues 1-135) of the u-PA molecule, more precisely within the cysteine-rich region termed the growth factor region as this region shows homologies to the part of epidermal growth factor (EGF) which is responsible for binding to the EGF receptor. The amino acid residues which appear to be critical for binding are located within the sequence 12-32 (Appella et al., 1987). Synthetic peptides have been constructed that inhibit the binding of very low (100 nM) concentrations. The lack of cross-reactivity between the murine and the human peptides indicates that the binding between u-PA and u-PAR is strongly species specific.

Brief Summary Text (16):
Binding of u-PA to u-PAR is specific in the sense that as yet no other protein has been found to compete for binding to the receptor, though several proteins structurally related to u-PA, including t-PA and plasminogen, have been tested (Stoppelli et al., 1985, Vassalli et al., 1985, Nielsen et al., 1988). Fragments of u-PA containing only the receptor binding domain, e.g. ATF, ensure specificity of the binding to the receptor, since other molecules that might bind u-PA (protease nexin and the specific plasminogen activator inhibitors PAI-1 and PAI-2) recognize the catalytically active region (Stoppelli et al., 1985; Nielsen et al., 1988). PAI-1 is able to form a covalent complex with u-PA but not with pro-u-PA (Andreasen et al., 1986).

Brief Summary Text (24):

In the intact organism, pro-u-PA is the predominant form of u-PA in intracellular stores, and it also constitutes a sizable fraction of the u-PA in extracellular fluids (Skriver et al., 1984, Kielberg et al., 1985). Extracellular activation of pro-u-PA may therefore be a crucial step in the physiological regulation of the u-PA pathway of plasminogen activation. The plasmin-catalyzed activation of pro-u-PA provides a positive feedback mechanism that accelerates and amplifies the effect of activation of a small amount of pro-u-PA. The initiation of the u-PA pathway of plasminogen activation under physiological conditions, however, involves triggering factors that activate pro-u-PA as described herein. Mutants of human single-chain pro-u-PA in which lysine 158 is changed to another amino acid (e.g. Glu or Gly) are not, or are only to a small extent, converted to active two-chain u-PA (Nelles et al., 1987).

Brief Summary Text (28):
Plasminogen, as well as plasmin, binds to many types of cultured cells, including thrombocytes, endothelial cells and several cell types of neoplastic origin (Miles and Plow, 1985, Hajjar et al., 1986, Plow et al., 1986, Miles and Plow 1987, Burtin and Fondaneche, 1988). The binding is saturable with a rather low affinity for plasminogen (K.sub.D 1 .mu.M). At least in some cell types, binding of plasmin appears to utilize the same site as plasminogen, but the binding parameters for plasmin indicate that more than one type of binding site for plasminogen and plasmin may exist. Thus, on some cell types, plasmin and plasminogen bind with almost equal affinity (Plow et al., 1986), while on others plasmin apparently binds with a higher affinity (K.sub.D 50 nM) than plasminogen (Burtin and Fondaneche, 1988). The binding is inhibited by low amounts of lysine and lysine analogues and appears to involve the kringle structure of the heavy chains of plasminogen and plasmin (Miles et al., 1988).

Brief Summary Text (40):
The new findings include the requirement, in the presence of serum, for binding of plasminogen, the ability of bound u-PA under these conditions to activate plasminogen, the presence of pro-u-PA on the cells, the ability of bound plasmin to activate pro-u-PA, and the ability of endogenous plasminogen activator inhibitor PAI-1, as well as added plasminogen activator inhibitor PAI-2, to regulate the surface plasminogen activation. By these means tumor cells can acquire the broad-spectrum proteolytic activity of plasmin, bound to their surface in such a way that it is protected from inactivation by serum protease inhibitors, and ideally situated to be employed in the degradation of the pericellular matrix.

Brief Summary Text (42):
Human tumor cells are very commonly found to secrete plasminogen activator of the urokinase type (u-PA). By this means they are able to recruit the proteolytic potential available in the high concentration of plasminogen in plasma and other body fluids. The invasive properties of tumor cells may be at least partly dependent on their proteolytic capability mediated through the broad spectrum of activity of plasmin and including its indirect actions in activating other latent proteases, such as collagenases. The expression of protease activity by tumor cells facilitates their penetration of basement membranes, capillary walls and interstitial connective tissues, allowing spread to other sites and establishment of metastases.

Brief Summary Text (48):
The purified protein could be chemically cross-linked with u-PA. Its amino acid composition and N-terminal sequence were determined (30 residues, some of which with some uncertainty). It was found to be heavily N-glycosylated, deglycosylation resulting in a protein with an apparent molecular weight of about 30-35 kD. The apparent molecular weight of u-PAR from different cell lines and from PMA-stimulated and non-stimulated U937 cells varied somewhat. This heterogeneity disappeared after deglycosylation and was thus due to differences in glycosylation of u-PAR from the various sources.

Brief Summary Text (52):
The deduced amino acid sequence indicated that u-PAR is produced as a 313 residues long protein with a 282 residues long hydrophilic N terminal part (probably extracellular) followed by 21 rather hydrophobic amino acids (probably a trans-membrane domain). The potential extracellular part is organised in 3 repeats

with striking homologies, particularly with respect to the pattern of cysteines. This may indicate the presence of distinct domains that may bind different ligands.

Brief Summary Text (56):
In Example 7 it is demonstrated that after incubation of monolayer cultures of human HT-1080 fibrosarcoma cells with purified native human plasminogen in serum containing medium, bound plasmin activity can be eluted from the cells with tranexamic acid, an analogue of lysine. The bound plasmin is the result of plasminogen activation on the cell surface; plasmin activity is not taken up onto cells after deliberate addition of plasmin to the serum containing medium. The cell surface plasmin formation is inhibited by an anti-catalytic monoclonal antibody to u-PA, indicating that this enzyme is responsible for the activation.

Brief Summary Text (58):
The binding and subsequent protection of plasmin was abolished by low concentrations of the lysine analogue, tranexamic acid. It is therefore likely that plasmin binding involves the lysine affinity sites situated in the heavy-chain kringles of plasmin. Plasmin released from the cells was partially inactivated in the serum medium. As long as the plasmin remained bound, it was protected from serum inhibitors but could be inhibited by aprotinin or an anti-catalytic monoclonal antibody.

Brief Summary Text (78):
The enzyme urokinase-type plasminogen activator (u-PA) has only one well-defined macromolecular substrate, namely plasminogen. By cleavage at Arg.sup.560, plasminogen is activated to the broad spectrum protease plasmin. By the term "preventing u-PA from converting plasminogen into plasmin" is therefore meant that this activation by u-PA is substantially inhibited or a situation where the activation is sufficiently inhibited so as to inhibit or reduce the undesired effect of the plasmin.

Brief Summary Text (120):
The present invention also relates to pure u-PAR. As mentioned above, pure u-PAR has been made for the first time in accordance with the present invention. Pure u-PAR in glycosylated form shows, in an SDS-PAGE at a load of approximately 1 .mu.g, substantially one and only one silver stained band having an apparent molecular weight in the range of about 55-60 kD. The presence of substantially one and only one silver stained band in this SDS-PAGE is a proof of the purity of the u-PAR. Another proof of the purity of the u-PAR is the presence of a single amino-terminal amino acid sequence in purified u-PAR preparations. While it has been found that different cells may produce u-PARs having different glycosylation, the glycosylated u-PARs, upon deglycosylation, were all found to have an identical electrophoretic mobility (corresponding to substantially one and only one band at about 30-35 kD in an SDS-PAGE), indicating that the peptide part of the molecule is identical in all cases.

Brief Summary Text (121):
As appears from the Examples, pure u-PAR in glycosylated form may be prepared from a biological material containing u-PAR by temperature-induced phase separation of detergent extracts followed by affinity chromatography purification with immobilized DFP-u-PA. The detergent is preferably a non-ionic detergent such as a polyethylene glycol ether, e.g. Triton X-114. The temperature was found to be relatively critical in the range of 34.degree.-40.degree. C., such as about 37.degree. C., for 10 minutes.

Brief Summary Text (124):
On the basis of the amino-terminal amino acid sequence of pure u-PAR, a 24-mer nucleotide probe was synthesized and used to screen a library to identify and isolate recombinant clones carrying the cDNA for u-PAR. The identity of the cDNA clones was confirmed by comparing the nucleotide sequence of this cDNA clone with the amino terminal sequence of the purified u-PAR, and by expressing said cDNA in mouse L cells and assaying their u-PA-binding properties.

Brief Summary Text (125):
The abbreviations of the amino acids used herein are the following:

Brief Summary Text (126):
One aspect of the invention relates to a polypeptide comprising a characteristic amino acid sequence derived from a u-PAR which polypeptide comprises at least 5 amino acids and up to the complete sequence of u-PAR as shown below as the DNA sequence and the deduced amino acid sequence of the clone p-uPAR-1 (hereinafter Sequence A. The signal peptide is underlined and the first 30 amino acids, the sequence of which has been determined on the purified protein with an Applied Biosystems gas phase sequencer (see Example 1), are overlined. The putative transmembrane domain is doubly underlined. The star symbols indicate the potential N-linked glycosylation sites. ##STR1## or an analogue thereof.

Brief Summary Text (127):
The invention relates to any polypeptide comprising at least 5 amino acids and up to the complete sequence of u-PAR from amino acid 1 to 313, and any analogue to such a polypeptide.

Brief Summary Text (129):
In the present context, the term "characteristic amino acid sequence derived from the u-PAR" is intended to mean an amino acid sequence, such as an epitope, which comprises amino acids constituting a substantially consecutive stretch (in terms of linear or spatial conformation) in u-PAR, or amino acids found in a more or less non-consecutive conformation in u-PAR, which amino acids constitute a secondary or tertiary conformation having interesting and useful properties, e.g. as therapeutics or diagnostics. Thus, amino acids present at different positions in u-PAR but held together e.g. by chemical or physical bonds, e.g. by disulphide bridges, and thereby forming interesting tertiary configurations are to be understood as "characteristic amino acid sequences". The characteristic amino acid sequence may comprise a consecutive subsequence of the amino acid sequence of u-PAR of greater or smaller length or a combination of two or more parts of such subsequences which may be separated by one or more amino acid sequences not related to u-PAR. Alternatively, the characteristic amino acid sequences may be directly bonded to each other.

Brief Summary Text (131):
The term "analogue" is used in the present context to indicate a protein or polypeptide of a similar amino acid composition or sequence as the characteristic amino acid sequence derived from the u-PAR, allowing for minor variations which do not have an adverse effect on the immunogenicity of the analogue. The analogous polypeptide or protein may be derived from mammals or may be partially or completely of synthetic origin.

Brief Summary Text (132):
The present invention also relates to a substantially pure polypeptide which is recognized by an antibody raised against or reactive with a polypeptide comprising the amino acid sequence defined above.

Brief Summary Text (133):
In the present context, the term "substantially pure" is understood to mean that the polypeptide in question is substantially free from other components, e.g. other polypeptides or carbohydrates, which may result from the production and/or recovery of the polypeptide or otherwise be found together with the polypeptide. The high purity of the polypeptide of the invention is advantageous when the polypeptide is to be used for, e.g., the production of antibodies. Also due to its high purity, the substantially pure polypeptide may be used in a lower amount than a polypeptide of a conventional lower purity for most purposes. The purification of the polypeptide of the invention may be performed by methods known to a person skilled in the art, but particularly the low concentrations of u-PAR in biological material and the strongly hydrophobic nature of the receptor has hitherto hampered its purification. Now, however, the combination of temperature-induced phase separation of detergent extracts of cells and affinity chromatography with immobilized DFP-treated u-PA has led to its successful purification in amounts high enough (100-200 .mu.g) to have enabled a partial amino acid sequencing and further characterization.

Brief Summary Text (137):
The polypeptide of the invention may also be a fusion protein in which characteristic amino acid sequences) from u-PAR is/are fused to another polypeptide

sequence. The polypeptide to which the characteristic amino acid sequence(s) from u-PAR is/are fused may be one which results in an increased expression of the protein when expressed in an organism, or facilitates or improves the purification and recovery of the fusion protein from said organism in terms of a more easy and economical recovery, or confers to the u-PAR the property of inhibiting u-PA (as it would be in the case of a u-PAR-PAI-1 fusion).

Brief Summary Text (138):
In some cases, it may be advantageous to cleave the fusion protein so as to obtain a polypeptide which substantially solely comprises characteristic amino acid sequence(s) from u-PAR. In these cases, the characteristic amino acid sequence(s) from u-PAR is/are preferably fused to a polypeptide sequence which may be specifically recognized by a cleaving agent, e.g. a chemical such as cyanogen bromide, hydroxylamine and 2-nitro-5-thiocyanobenzoate, or an enzyme, e.g. a peptidase, proteinase or protease, e.g. trypsin, chlostripain, and staphylococcal protease or factor Xa.

Brief Summary Text (148):
The DNA fragment of the invention may comprise a nucleotide sequence encoding a polypeptide fused in frame to the nucleotide sequence encoding the characteristic amino acid sequence with the purpose of producing a fused polypeptide. When using recombinant DNA technology, the fused sequence may be inserted into an appropriate vector which is transformed into a suitable host organism. Alternatively, the DNA fragment of the invention may be inserted in the vector in frame with a gene carried by the vector, which gene encodes a suitable polypeptide. The host organism, which might be of eukaryotic or prokaryotic origin, for instance a yeast or a mammalian cell line, is grown under conditions ensuring expression of the fused sequence after which the fused polypeptide may be recovered from the culture by physico-chemical procedures, and the fused polypeptide may be subjected to gel filtration and affinity chromatography using an antibody directed against the antigenic part(s) of the fused polypeptide. After purification, the polypeptide of the invention and the polypeptide to which it is fused may be separated, for instance by suitable proteolytic cleavage, and the polypeptide of the invention may be recovered, e.g. by affinity purification or another suitable method.

Brief Summary Text (150):
The DNA fragment described above may be obtained directly from genomic DNA or by isolating mRNA and transferring it into the corresponding DNA sequence by using reverse transcriptase producing cDNA. When obtaining the DNA fragment from genomic DNA, it is derived directly by screening for genomic sequences, hybridizing to a DNA probe prepared on the basis of the full or partial amino acid sequence of u-PAR. When the DNA is of complementary DNA (cDNA) origin, it may be obtained by preparing a cDNA library on the basis of mRNA from cells containing a u-PAR or parts thereof. Hybridization experiments may then be carried out using synthetic oligonucleotides as probes to identify the cDNA sequence encoding the u-PAR or part thereof. cDNA differs from genomic DNA in, e.g. that it lacks certain transcriptional control elements and introns which are non-coding sequences within the coding DNA sequence. These elements and introns are normally contained in the genomic DNA. The DNA fragment may also be of synthetic origin, i.e. prepared by conventional DNA synthesizing method, e.g. by using a nucleotide synthesizer. The DNA fragment may also be produced using a combination of these methods.

Brief Summary Text (157):
The present invention also relates to a method of producing the polypeptides described above. Suitably, the polypeptides are prepared using recombinant DNA-technology e.g. the methods disclosed in Maniatis et al. op. cit. More specifically, the polypeptides may be produced by a method which comprises cultivating or breeding an organism carrying a DNA-fragment encoding a characteristic amino acid sequence from an u-PAR, e.g. the above described DNA fragment, under conditions leading to expression of said DNA fragment, and subsequently recovering the polypeptide from the organism.

Brief Summary Text (158):
As described above, the organism which is used for the production of the polypeptide may be a higher organism, e.g. an animal, or a lower organism, e.g. a microorganism.

Irrespective of the type of organism employed for the production of the polypeptide, the DNA fragment encoding the characteristic amino acid sequence from an u-PAR should be introduced in the organism. Conveniently, the DNA fragment is inserted in an expression vector, e.g. a vector as defined above, which is subsequently introduced into the host organism. The DNA fragment may also be directly inserted in the genome of the host organism. The insertion of the DNA fragment in the genome may be accomplished by use of a DNA fragment as such or cloned in bacteria, phage lambda or other vectors, carrying the DNA fragment and being capable of mediating the insertion into the host organism genome. The insertion of the DNA fragment into an expression vector or into the genome of the host organism may be accomplished as described e.g. by Colbere-Garapin F. et al., J. Molec. Biol., 150; 1-14 (1981): A New Dominant Hybrid Selective Marker for Higher Eucaryotic Cells.

Brief Summary Text (161):
When the polypeptide of the invention comprises one or more distinct parts, e.g. being a fusion protein comprising on the one hand characteristic amino acid sequence(s) from u-PAR and on the other hand amino acid sequence(s) constituting a polypeptide which is not related to u-PAR, the DNA fragments encoding each of these polypeptides may be inserted in the genome or expression vector separately or may be coupled before insertion into the genome or expression vector by use of conventional DNA techniques such as described in Maniatis et al. op. cit.

Brief Summary Text (169):
c) Preparative electrophoresis procedures; for instance the following procedure: A supernatant from a centrifuged enzyme treated cell or cell line preparation is subjected to a gel electrophoresis, such as a sodium dodecyl sulphate-polyacrylamidgel electrophoresis (SDS-PAGE) (cf. Laemmli, U.K. Nature, 227:680-685; 1970), or an agarose gel electrophoresis. Subsequently, labelled antibodies, such as monoclonal antibodies, reactive with u-PAR, are used to identify bands primarily constituted by the isolated u-PAR compounds. For instance, the antibodies may be used in any conventional immunoblotting technique. The markers may be isotopes or fluorescein labels detectable by means of relevant sensitive films. After identification, the u-PAR containing bands of the gel may be subjected to a treatment resulting in the release of the u-PAR compounds from the gels, such as procedures involving slicing up the gel and subsequent elution of u-PAR compounds. Optionally, the amino acid sequence of the u-PAR proteins obtained may be determined.

Brief Summary Text (171):
Prior to cultivation of the microorganism, the DNA fragment encoding the polypeptide of the invention may be subjected to modification, before or after the DNA fragment has been inserted in the vector. The polypeptide produced may also be subjected to modification. The modification may comprise substitution, addition, insertion, deletion or rearrangement of one or more nucleotides and amino acids in the DNA fragment and the polypeptide, respectively, or a combination of these modifications. The term "substitution" is intended to mean the replacement of any one or more amino acids or nucleotides in the full amino acid or nucleotide sequence with one or more others, "addition" is understood to mean the addition of one or more amino acids or nucleotides at either end of the full amino acid or nucleotide sequence, "insertion" is intended to mean the introduction of one or more amino acids or nucleotides within the full amino acid or nucleotide sequence, and "deletion" is intended to indicate that one or more amino acids or nucleotides have been deleted from the full amino acid or nucleotide sequence whether at either end of the sequence or at any suitable point within it. "Rearrangement" is intended to indicate that one or more amino acids or nucleotides or the sequence has been exchanged with each other. The DNA fragment may, however, also be modified by subjecting the organism carrying the DNA fragment to mutagenization, preferably site directed mutagenization so as to mutagenize said fragment. When the organism is a microorganism, the mutagenization may be performed by using conventional mutagenization means such as ultraviolet radiation, ionizing radiation or a chemical mutagen such as mitomycin C, 5-bromouracil, methylmethane sulphonate, nitrogen mustard or a nitrofuran or mutagens known in the art, e.g. mutagens of the type disclosed in Miller, J. H., Molecular genetics, Unit III, Cold Spring Harbor Laboratory 1972.

Brief Summary Text (172):

Examples of suitable modifications of the DNA sequence are nucleotide substitutions which do not give rise to another amino acid sequence of the protein, but which, e.g., correspond to the codon usage of the specific organism in which the sequence is inserted; nucleotide substitutions which give rise to a different amino acid sequence and therefore, possibly, a different protein structure without, however, impairing the critical properties of the polypeptide encoded by the DNA sequence; a subsequence of the DNA sequence shown above encoding a polypeptide which has retained the receptor properties of the native u-PAR; or a DNA sequence hybridizing to at least part of a DNA prepared on the basis of the DNA sequence shown above, provided that it encodes a polypeptide which has the biological property of u-PAR.

Brief Summary Text (174):
It is well-known that use of recombinant DNA-techniques, including transgenic techniques, may be associated with another kind of processing of the polypeptide than the processing of the polypeptide when produced in its natural environment. Thus, when a bacterium such as E. coli is used for the production of the polypeptide of the invention, the amino acid residues of the polypeptide are not glycosylated, whereas the polypeptide may be glycosylated when produced in another microorganism or organism.

Brief Summary Text (176):
The term "truncated polypeptide" refers to a polypeptide deleted for one or more amino acid residues eventually resulting in changing of the properties of the polypeptide, e.g. solubility. In a further meaning, the term "truncated polypeptide" refers to a mixture of polypeptides all derived from one polypeptide or expressed from the gene encoding said polypeptide. Such truncated polypeptides might arise for instance in vector/host cell systems in which part of the cDNA has been deleted by restriction enzyme digestion or other suitable methods, resulting in the expression of a protein not normally produced in that system.

Brief Summary Text (177):
Also, the polypeptide of the invention may be prepared by the well-known methods of liquid or solid phase peptide synthesis utilizing the successive coupling of the individual amino acids of the polypeptide sequence or the coupling of individual amino acids forming fragments of the polypeptide sequence which fragments subsequently are coupled so as to result in the desired polypeptide. The solid phase peptide synthesis may e.g. be performed as described by R. B. Merrifield, J. Am. Chem. Soc. 85, 1963, p. 2149. In solid phase synthesis, the amino acid sequence is constructed by coupling an initial amino acid to a solid support and then sequentially adding the other amino acids in the sequence by peptide bonding until the desired length has been obtained. In this embodiment, the solid support may also serve as the carrier for the polypeptide of the invention in a vaccine preparation as described below. The preparation of synthetic peptides may be carried out essentially as described in Shinnick, Ann. Rev. Microbiol. 37, 1983, pp. 425-446.

Brief Summary Text (190):
The diagnostic agent, may, e.g, be an antibody as defined above. Alternatively, the diagnostic agent may be in the form of a test kit comprising in a container a polypeptide comprising a characteristic amino acid sequence of u-PAR, e.g. a sequence including or included in the sequence (1). The diagnostic agent may be used in the diagnosis of diseases related to abnormal numbers of u-PARs residing on the cell.

Brief Summary Text (192):
Examples of enzymes useful as labels are .beta.-galactosidase, urease, glucose oxidase, carbonic anhydrase, peroxidases (e.g. horseradish peroxidase), phosphatases (e.g. alkaline or acid phosphatase), glucose-6-phosphate dehydrogenase and ribonuclease.

Brief Summary Text (193):
Enzymes are not in themselves detectable, but must be combined with a substrate to catalyze a reaction the end product of which is detectable. Thus, a substrate may be added to the reaction mixture resulting in a coloured, fluorescent or chemiluminescent product or in a colour change or in a change in the intensity of the colour, fluorescence or chemiluminescence. Examples of substrates which are

useful in the present method as substrates for the enzymes mentioned above are H.sub.2 O.sub.2, p-nitrophenylphosphate, lactose, urea, .beta.-D-glucose, CO.sub.2, RNA, starch, or malate. The substrate may be combined with, e.g. a chromophore which is either a donor or acceptor.

Brief Summary Text (197):
In an embodiment of the invention an antibody or a polypeptide of the invention may be coupled to a bridging compound coupled to a solid support. The bridging compound, which is designed to link the solid support and the antibody may be hydrazide, Protein A, glutaraldehyde, carbodiimide, or lysine.

Brief Summary Paragraph Table (1):

Three-letter abbreviation	One-letter symbol	Amino acid
Ala	A	Arginine
Arg	R	Asparagine
Asn	N	Aspartic acid
Asp	D	Asparagine or aspartic acid
Asx	B	Cysteine
Cys	C	Glutamine
Gln	Q	Glutamic acid
Glu	E	Glutamine or glutamic acid
Glx	Z	Glycine
Gly	G	Histidine
His	H	Isoleucine
Ile	I	Leucine
Leu	L	Lysine
Lys	K	Methionine
Met	M	Phenylalanine
Phe	F	Proline
Pro	P	Serine
Ser	S	Threonine
Thr	T	Tryptophan
Trp	W	Tyrosine
Tyr	Y	Valine
Val	V	

Drawing Description Text (3):
FIG. 1A) The Triton X-114 fraction containing membrane proteins from PMA-treated U937a cells was subjected to affinity chromatography using immobilized DFP-treated u-PA. The neutralized column eluate was dialyzed aginst 0.1% acetic acid and concentrated by lyophilization. A portion, representing 2.times.10.sup.8 cells before purification, was run on 6-16% gradient SDS-PAGE under reducing conditions (lane 1). The gel was silver-stained. The molecular weights of marker proteins (lane 2) are indicated.

Drawing Description Text (11):
FIGS. 7A-C. FIG. A shows the initial amino-terminal amino acid sequence information and the oligonucleotide synthesized and used for library screening; I stands for inosine. FIG. 7B shows the restriction map of p-uPAR-1 clone and the strategy employed for the complete double stranded sequence. FIG. 7C shows the hydrophobicity plot. The abscissa shows the amino acid residue position, the ordinate the degree of hydrophobicity calculated using the algorithm of Hopp and Wood (1981) and Kyte and Doolittle (1982).

Drawing Description Text (16):
FIG. 11 shows SDS-PAGE (12.5%) electrophoretic analysis of the p-u-PAR-PFLM-1 mutant transfected into LB6 cells. Cells were incubated with iodinated ATF, washed, extracted with Triton X-114, and an amount of extract corresponding to 300,000 cells cross-linked with DSS as described before and run on the gel (right-hand panel). Similarly, conditioned medium was centrifuged at 100,000.times.g, and the supernatant (a volume corresponding to 15,000 cells) was incubated with iodinated ATF, cross-linked with DSS, and analyzed by SDS-PAGE (left-hand panel). Lanes a and b are duplicates from cells grown at different densities.

Drawing Description Text (17):
FIG. 12 shows an elution profile from cation-exchange chromatography of amino acids released from u-PAR after acid hydrolysis. The protein was initially purified from PMA-stimulated U937 cells (6.times.10.sup.9 cells) by Triton X-114 detergent-phase separation and affinity chromatography (DFP-u-PA Sepharose). To improve purity and eliminate interference on amino acid analysis from low molecular weight compounds, this receptor preparation was dialysed thoroughly against 0.1% acetic acid, lyophilized and then subjected to Tricine-SDS-PAGE followed by electrotransfer onto a 0,45 .mu.m PVDF-membrane (8 cm.times.8 cm). The insert shows the immobilized u-PAR after staining with Coomassie Brilliant Blue R-250. A slight decrease in mobility of u-PAR was observed in this experiment, due to a large excess of the zwitterionic detergent CHAPS in the lyophilized preparation. The stained area of the PVDF-membrane representing u-PAR was excised and hydrolysed in vacuo for 20 hours at 110.degree. in the presence of 3,3'-dithiodipropionic acid (DTDPA). Cys-X is the product formed between cysteine and DTDPA during hydrolysis, GlcN is glucosamine and EtN is ethanolamine.

Drawing Description Text (20):
FIGS. 15A-15C show the change in hydrophobic properties of purified u-PAR upon treatment with PI-PLC. u-PAR, purified from PMA-stimulated U937 cells, were either untreated (NONE) or incubated for 30 min at 37.degree. C. in 50 mM triethylamine/HCl (pH 7.5), 5 mM EDTA and 0.1% Triton X-100 without any phospholipases (MOCK) or in the presence of 20 .mu.l/ml PI-PLC (PI-PLC). One sample was incubated with 200 .mu.g/ml phospholipase D purified from cabbage in 50 nM acetate (pH 6.0), 10 mM CaCl.sub.2 (PLD), and another with 100 .mu.g/ml phospholipase A.sub.2 purified from bee venom in 50 mM HEPES (pH 8,0), 10 mM CaCl.sub.2 (PLA.sub.2).

Drawing Description Text (21):
These u-PAR preparations were then subjected to temperature-induced detergent-phase separation in 1% Triton X-114. This phase separation was repeated once for the resulting aqueous and detergent phases by addition of extra Triton X-114 and 0.1M Tris (pH 8.1), respectively. Finally, cross-linking analysis with 1 nM .sup.125 I-labelled ATF was performed on parallel aliquots of aqueous (A) and detergent (D) phases, followed by SDS-PAGE (10% T and 2.5% C) under non-reducing conditions. Areas corresponding to .sup.125 I-ATF/u-PAR complexes (Mr 70,000) were excised from the polyacrylamide gel and the radioactivity was determined (shown as % of total radioactivity in A+D at the bottom of each lane).

Drawing Description Text (22):
FIG. 16 shows a comparison of COOH-terminal amino acid sequences from proteins, in which the processing sites during GPI-membrane anchoring are known, to that predicted for u-PAR (SEQ ID NO:21) (based on amino acid analysis, Table 5). The amino acids involved in attachment to the glycolipid are highlighted. VSG (SEQ ID NO:17) and PARP (SEQ ID NO:16) refers to variant surface glycoprotein (and procyclic acidic repetitive protein from Trypanosoma brucei. CEA (SEQ ID NO:19) is carcinoembryonic antigen; PLAP (SEQ ID NO:18) is placental alkaline phosphatase and Thy-1 (SEQ ID NO:20) refers to the surface glycoprotein isolated from rat thymocytes.

Drawing Description Text (24):
FIG. 18 shows SDS-PAGE of detergent phase from Triton X-114 phase-separated extracts from U937 cells treated with PMA for different time periods, chemical cross linked to .sup.125 I-ATF. Non-treated cells and PMA (150 nM) treated cells were acid treated and lysed. The detergent phases were incubated with .sup.125 I-ATF, cross linked with DSS and run in a 6-16% SDS-PAGE gradient gel followed by autoradiography. Electrophoretic mobility of molecular weight standard proteins are indicated to the left. 1. Non-treated cells, 2. +PMA 3 hours, 3. +PMA 9 hours, 4. +PMA 24 hours, 5. +PMA 48 hours, 6. Blind, 7. 1% Triton X-114 total lysate (diluted 1/25) from HEp2 cells.

Drawing Description Text (26):
FIG. 20 shows SDS-PAGE of detergent phase from Triton X-114 phase-separated extracts from U937 cells treated with Dibuturyl cAMP for different time periods, chemical cross-linked to .sup.125 I-ATF. Non-treated cells and Dibuturyl cAMP (1 mM) treated cells were acid treated and lysed as described in Materials and Methods. The detergent phases were incubated with .sup.125 I-ATF, cross linked with DSS and run in a 6-16% SDS-PAGE gradient gel followed by autoradiography. Electrophoretic mobility of molecular weight standard proteins are indicated to the left. 1. Non-treated cells, 2. +Dibuturyl cAMP 12 hours, 3. +Dibuturyl cAMP 24 hours, 4: +Dibuturyl cAMP 48 hours, 5. +Dibuturyl cAMP 72 hours.

Drawing Description Text (28):
FIG. 22. Dependence of plasmin formation in serum medium on the concentration of added native human plasminogen. Confluent layers of HT-1080 cells were incubated for 3 hours in MEM medium (0.5 ml) containing 10% heat-inactivated and plasminogen-depleted fetal calf serum, with the addition of native human plasminogen to the concentrations shown. The conditioned media were harvested and the cells rinsed three times with PBS. The cells were treated with 1 mM tranexamic acid in PBS to obtain the bound fraction of plasmin. Plasmin was assayed in the cell-bound fraction (.smallcircle.--.smallcircle.) and the medium (.circle-solid.--.circle-solid.) as thioesterase activity.

Drawing Description Text (30):
FIG. 24. Plasmin release from HT-1080 cells into serum-free and serum-containing media. Confluent layers of HT-1080 cells were first loaded with plasmin by incubation for 1 hour at 37.degree. C. in serum-free MEM medium (0.5 ml) containing human plasmin (0-5 .mu.g/ml). After rinsing the cell layers three times, they were incubated for 2 hours at 37.degree. C. with either serum-free medium (.smallcircle.--.smallcircle.), medium containing 10% heat-inactivated and plasminogen-depleted fetal calf serum (.circle-solid.--.circle-solid.), or the latter with tranexamic acid (100 .mu.M) (.box-solid.--.box-solid.). Plasmin was then assayed in the cell-bound fraction (A) and the media (B). At the time of transfer to new media, there was approximately 28 ng of plasmin bound to the cells from the pretreatment with 2.5 .mu.g of plasmin/well.

Drawing Description Text (31):
FIG. 25. Effect of pretreatment of HT-1080 cells with DFP-u-PA on bound u-PA activity and ability to produce bound plasmin in serum medium. Confluent cell layers of HT-1080 cells were preincubated for 18 hours at 37.degree. C. with the concentrations shown of DFP-u-PA in serum-containing medium (0.5 ml). After rinsing three times, the cells were incubated for 1 hour at 37.degree. C. with MEM medium containing 10% heat-inactivated and plasminogen-depleted fetal calf serum, with addition of native human plasminogen (40 .mu.g/ml). After incubation, half the replicate wells were rinsed and treated with acid-glycine to recover the total bound u-PA (.smallcircle.--.smallcircle.) which now included DFP-u-PA, pro-u-PA and active u-PA. The other wells were used to recover bound plasmin (.circle-solid.--.circle-solid.) by elution with tranexamic acid.

Drawing Description Text (32):
FIG. 26. Activation of cell-bound u-PA proenzyme in serum medium after addition of plasminogen. Confluent layers of HT-1080 cells were prelabelled for 5 hours at 37.degree. C. with .sup.35 S-methionine. After restoring complete medium with 10% heat-inactivated and plasminogen-depleted fetal calf serum, native human plasminogen (50 .mu.g/ml) was added and the incubation continued for another 3 hours. Aprotinin (200 KIU/ml) was added before harvest of medium, and the rinsed cells were treated with acid-glycine to recover the bound u-PA fraction. Acid eluates were neutralized and immunoprecipitated with goat antibodies to u-PA, before SDS-PAGE under reducing conditions. The fluorogram shows: in lane 1, control immunoprecipitate of culture without plasminogen with goat antibodies to human t-PA; lane 2, culture without plasminogen immunoprecipitated with goat anti-u-PA antibodies; lane 3, culture with plasminogen immunoprecipitated with u-PA antibodies.

Drawing Description Text (33):
FIG. 27. Activation of cell-bound u-PA proenzyme in serum medium after the addition of plasminogen. Confluent layers of HT-1080 cells were incubated with MEM medium containing 10% heat-inactivated and plasminogen-depleted fetal calf serum and native human plasminogen (40 .mu.g/ml). After the time intervals shown, aprotinin (200 KIU/ml) was added and the rinsed cells were treated with acid-glycine to recover the bound fraction of u-PA. The u-PA in the neutralized eluate was assayed by an immunocapture method, using an NPGB inactivation step to determined the pro-u-PA index (see Methods). FIG. 27A shows the pro-u-PA index for cultures without (.smallcircle.--.smallcircle.) and with (.circle-solid.--.circle-solid.) plasminogen. The zero-time sample with plasminogen shows that some change already occurred during work-up of the cells. FIG. 27B shows the eluted u-PA activity from cultures without plasminogen (.smallcircle.--.smallcircle.), with plasminogen (.circle-solid.--.circle-solid.), and with plasminogen and a neutralizing monoclonal antibody to human PAI-1 (10 .mu.g/ml) (.box-solid.--.box-solid.).

Drawing Description Text (34):
FIG. 28. Model for cell surface plasminogen activation. In this proposed model, u-PA receptors (u-PA-R) and plasminogen receptors (plg-R) are depicted on the cell membrane. Before exposure to plasminogen (plg), virtually all the bound u-PA is present as pro-u-PA (open squares), but it is assumed that some active u-PA molecules exist (closed squares). On plasminogen (open rectangles) binding (which may be precluded by the presence of tranexamic acid), plasmin (pl, closed rectangles) is formed on the cell by the action of the bound active urokinase. This step may be inhibited by PAI-1 and PAI-2, and by an anti-catalytic monoclonal

antibody to u-PA (anti-u-PA-ab). The bound plasmin thus formed is resistant to inhibition by the alpha-2-anti-plasmin present in the serum medium, but sensitive to inhibition by aprotinin and an anti-catalytic monoclonal antibody to plasmin (anti-pl-ab). As active plasmin becomes available, it catalyzes the activation of more bound pro-u-PA to active u-PA, thus amplifying the proteolytic system. Activation of pro-u-PA is inhibited by tranexamic acid (which prevents plasminogen binding), aprotinin and an anti-catalytic monoclonal antibody to plasmin.

Drawing Description Text (56):
FIG. 45. Western blot showing the reactivity of the antisera used. 500 ng of purified u-PAR (lanes 2 and 4) or the Triton X-114 detergent phase obtained from 2.5.times.10.sup.6 PMA-stimulated U937 cells (lanes 1 and 3) were analyzed by SDS-PAGE under reducing conditions on a 6-16% gradient gel, and Western blotting using as the primary antisera mouse anti-u-PAR serum diluted 1:250 (lanes 1 and 2) or the above control serum at the same dilution (lanes 3 and 4).

Drawing Description Text (57):
FIGS. 46A-B show a Western blot, demonstrating the reactivity of polyclonal rabbit antibody against u-PAR. 75 .mu.l samples of Triton X-114 detergent phase from lysates of PMA-stimulated U937 cells were analyzed alone (lane 1), after mixing with DFP-treated u-PA (Example 1; final concentration 10 .mu.g/ml) (lane 4), or after mixing with the same amount of DFP-treated u-PA, followed by chemical cross-linking (lane 3). As a control, the same amount of DFP-treated u-PA was analyzed alone, after the performance of cross-linking (lane 5), or directly (lane 6). The sample in lane 2 contained 75 .mu.l of the cell lysate detergent phase, which was subjected to chemical cross-linking without the addition of DFP-treated u-PA. The samples were run on 6-16% gradient SDS-PAGE under non-reducing conditions, followed by electroblotting onto nitrocellulose. The sheets were incubated with purified and absorbed IgG from rabbit anti-u-PAR serum (FIG. 46A), or with purified and absorbed IgG from pre-immune serum from the same rabbit (FIG. 46B). The IgG concentration during the incubation was 12 .mu.g/ml in both cases. The sheets were developed with alkaline phosphatase-coupled antibody against rabbit IgG, followed by detection of alkaline phosphatase activity.

Detailed Description Text (6):
Tricine-SDS-PAGE of samples to be electroblotted for amino acid analysis or NH.sub.2 -terminal amino-acid sequencing was performed in a Mini Protean II apparatus (BioRad) according to Schagger and von Jagow, 1987, on a 0.75 mm homogeneous 7.7% T, 3% C gel. The gel was pre-electrophoresed for 3 hours at 15 mA in the gel buffer with 12 mM 3-mercaptopropanoic acid added as a scavenger. The freeze-dried sample was dissolved directly in 50 .mu.l of the sample buffer with 40 mM dithioerythritol as the reducing agent, and boiled for 2 minutes. The gel buffer used for pre-electrophoresis was replaced with electrophoresis buffer, after which electrophoresis was performed for 4 hours at 60 V.

Detailed Description Text (7):
Electroblotting of samples for amino acid analysis or NH.sub.2 -terminal amino acid sequencing. After electrophoresis, the Tricine-SDS-polyacrylamide gel was electroblotted onto a polyvinylidene difluoride (PVDF) membrane (Millipore), using a semi-dry electroblotting apparatus (JKA Instruments, Denmark). Electroblotting took place at pH 11.0 in 10 mM CAPS (3-(cyclohexylamino)-1-propanesulfonic acid), including 0.4 mM dithioerythritol and 10% methanol, and was performed at 0.8 mA/cm.sup.2 for 2 hours. The protein was localized by staining with Coomassie R250 for 2 minutes and brief destaining, followed by wash in water (Matsudaira, 1987).

Detailed Description Text (8):
Alkylation of electroblotted protein and amino acid sequencing. The Coomassie-stained protein band was cut out from the PVDF-membrane and treated with 25 mM iodoacetamide in 50 mM sodium borate, pH 8.0, for 1 hour in the dark at room temperature. After the reaction, it was washed extensively with water and dried under argon. The protein on the dried filter was sequenced on an Applied Biosystems protein sequencer, model 477A. The on-line HPLC identification system for the PTH amino acid derivatives included the derivative of carboxymethylcysteine (produced by deamidation of the amidomethyl derivative during conversion). The correct identification of this derivative was assured by a test-sequencing of chicken

lysozyme (with cysteine at residue no. 6) after parallel preparative electrophoresis, electroblotting and alkylation.

Detailed Description Text (9):
Determination of amino acid composition and amino sugars. For hydrolysis of electroblotted u-PAR, areas of PVDF membranes containing Coomassie-stained and in situ alkylated protein were treated with 6M HCl containing 0.05% phenol for 20 h in vacuo at 110.degree. C. Amino acid analysis was performed on a Waters amino acid analyzer equipped with a post-column o-phthaldialdehyde identification system, as described (Barkholt and Jensen, 1989).

Detailed Description Text (14):
Cell lysis and detergent phase separation. PMA-stimulated U937a cells were washed and acid-treated as described by Nielsen et al., 1988. 20 ml lysis buffer (0.1M Tris/HCl, pH 8.1, 1% Triton X114, 10 mm EDTA, 10 .mu.g/ml Aprotinin) and 0.2 ml 100 mM phenylmethylsulfonylfluoride in dimethylsulfoxide were added to 10.sup.9 acid-treated cells at 0.degree. C. The suspension was mixed thoroughly, left on ice for 5 minutes, mixed again and left at 0.degree. C. for another 5 minutes, after which it was clarified by centrifugation at 4.degree. C., 16,000.times.g for 10 minutes.

Detailed Description Text (17):
Lysates and detergent phases from other cell types (as indicated) were prepared in the same manner, except that smaller amounts of cell material were used. The amounts of all reagents were reduced proportionally. In one experiment, 0.5% CHAPS was used as the lysis detergent instead of 1% Triton X114. In that experiment, no phase separation was performed.

Detailed Description Text (18):
Preparation of affinity matrix. 2.5.times.10.sup.6 IU (approximately 25 mg) of u-PA (Serono) was dissolved in 25 ml 0.1M Tris/HCl, pH 8.1, 0.1% Tween 80. The enzyme was inactivated by addition of 250 .mu.l of a fresh 500 mM stock solution of diisopropylfluorophosphate (DFP) in isopropanol and incubation for 4 hours at 37.degree. C., with a further addition of the same amount of DFP after the first 2 hours.

Detailed Description Text (19):
The reaction was stopped by extensive dialysis at 0.degree. C. against 0.25M $NaHCO_3$, 0.5M NaCl, 0.1% Triton X-100, pH 8.5.

Detailed Description Text (21):
Affinity purification. The clarified detergent fraction obtained from 6.times.10.sup.9 U937a cells was diluted with 1 vol washing buffer-1 (10 mM sodium phosphate, 140 mM sodium chloride, 0.1% CHAPS, pH 7.4) and chromatographed on a column containing 8 ml DFP-u-PA-Sepharose, equilibrated with the same buffer. After application of the sample, the column was washed with washing buffer-1, followed by washing buffer-2 (10 mM sodium phosphate, 1M sodium chloride, 0.1% CHAPS, pH 7.4). The column was eluted from below with elution buffer (0.1M acetic acid, 0.5M sodium chloride, 0.1% CHAPS, pH 2.5). Elution fractions were immediately titrated to pH 7.5 by addition of the appropriate volume of 0.1M sodium phosphate, 1.0M sodium carbonate, pH 9.0. u-PAR-containing fractions were identified by chemical cross-linking to the .sup.125 I-labelled amino terminal (ATF) fragment of urokinase, followed by SDS-PAGE and autoradiography. Purified u-PAR samples for amino acid analysis or NH_2 -terminal amino acid sequencing were dialyzed against 0.1% acetic acid and lyophilized.

Detailed Description Text (22):
Protein labelling with .sup.125 I. .sup.125 I-labelling of ATF was performed as described previously (Nielsen et al., 1988), except that 0.1% Triton X100 was replaced by 0.01% Tween 80. Purified u-PAR, concentrated by freeze-drying after dialysis against 0.1% acetic acid, was iodinated in the same manner, except that 1.5 .mu.g protein was treated with 250 .mu.Ci .sup.125 I in a volume of 25 .mu.l.

Detailed Description Text (26):
For complete removal of N-bound carbohydrate, the samples were denatured under

mildly reducing conditions by the addition of SDS and dithiothreitol to final concentrations of 0.5% and 1.6 mM, respectively, and boiling for 3 minutes. Aliquots of the denatured samples (10 .mu.l) were adjusted to include 200 mM sodium phosphate, pH 8.6, 1.5% Triton X-100, 10 mM 1,10 phenanthroline (added from a methanol stock solution) and either 1 unit of peptide:N-glycosidase F (N-glycanase, Genzyme), or no enzyme, in a total volume 30 .mu.l. Deglycosylation was performed at 37.degree. C. for 20 hours. During studies on non-fractionated cell lysates obtained after lysis with CHAPS, 100 mM .beta.-mercaptoethanol was used for reduction instead of dithiothreitol, and 10 mM EDTA was included during deglycosylation instead of 1,10 phenanthroline.

Detailed Description Text (29):
Purification. PMA-stimulated U937a cells were acid-treated to remove any surface-bound u-PA and lysed in a Triton X114 containing buffer. The detergent extract was subjected to temperature-induced phase separation, and the isolated detergent phase was used as the raw material for affinity chromatography. The acid eluates were neutralized and analyzed, either directly or after concentration by dialysis against 0.1% acetic acid and lyophilization. The electrophoretic appearance of the purified material is shown in FIGS. 1A-C.

Detailed Description Text (33):
Quantification by amino acid analysis indicated a purification yield of 6-9 .mu.g polypeptide (corresponding to about 10-15 .mu.g u-PAR glycoprotein; see below) from 6.times.10.sup.9 cells.

Detailed Description Text (34):
Amino acid composition and NH.sub.2 -terminal amino acid sequences. The amino acid composition of the purified protein after preparative electrophoresis, electroblotting and alkylation with iodoacetamide is shown in table 1. This composition includes a strikingly high content of cysteine residues. Further, it is noted that rather few lysine residues are present. The analysis system employed allows the quantification of glucosamine and galactosamine in addition to the amino acids. Glucosamine was detected in an amount corresponding to approximately 30 mol of N-acetylglucosamine per mol protein, correcting for loss during hydrolysis. In contrast, no galactosamine was identified.

Detailed Description Text (35):
The high number of glucosamine residues detectable after acid hydrolysis, as well as the large decrease in apparent molecular mass following treatment with peptide:N-glycosidase F (see below), indicate that large side chains of N-linked carbohydrate are present in the protein. The failure to detect any galactosamine indicates that this type of O-linked carbohydrate is absent in u-PAR. However, the presence of other O-linked oligosaccharides that escape detection by amino acid analysis cannot be excluded.

Detailed Description Text (36):
Two amino acid sequencing experiments were performed. In the first sequencing experiment, direct NH.sub.2 -terminal sequencing of affinity-purified u-PAR was performed after dialysis and lyophilization. A partial sequence (Table 2A) was obtained, and it was demonstrated that only one sequence was present in the purified material.

Detailed Description Text (38):
As seen in Table 2, all amino acid residues identified proved identical when comparing the two sequences. Furthermore, positions 3, 6 and 12, which were identified only in the second experiment, all proved to be cysteines. Thus, the lack of any identification at these positions in the first experiment was to be ascribed to the lack of alkylation. It was clear that the only detectable NH.sub.2 -terminal sequence in the preparation was associated with the electrophoretic mobility of u-PAR. Consequently, no additional sequences were hidden in the form of, for example, low molecular weight peptide components associated with the major polypeptide chain.

Detailed Description Text (39):
A search in the Georgetown University protein data base did not reveal any identity,

nor even pronounced homology, of the u-PAR NH.sub.2 -terminal amino acid sequence to any known protein.

Detailed Description Text (40):
The amino terminus, like the amino acid composition of the entire protein, is rich in cysteine residues.

Detailed Description Text (41):
Data for probe construction (Example 2) were derived from the sequencing shown in Table 2A. For this construction, position 6 of the amino acid sequence was tentatively assigned Asn; see footnote a of Table 2A.

Detailed Description Text (59):
Samples to be analyzed by chemical cross-linking to .sup.125 I-ATF were 50-fold diluted in 0.1M Tris/HCl, 1% Triton X-114, pH 8.1. The diluted samples were either clarified by addition of 0.25% w/v CHAPS (final concentration) or subjected to a single round of temperature induced phase separation (see Example 1). After the phase separation of 1 vol. of diluted sample, each phase (i.e., the detergent and buffer fraction, respectively) was made up to 1 vol. by addition of 0.1M Tris/HCl, pH 8.1, and clarified by addition of 0.25% CHAPS (final concentration).

Detailed Description Text (61):
Enzymatic deglycosylation with N-Glycanase (Genzyme) was performed according to example 1, except that the actual concentrations during the deglycosylation step were the following: 0.08% SDS; 0.26 mM dithiothreitol; 0.11M sodium phosphate; 0.9% Triton X-100; 5.3 mm 1,10 phenanthroline; 33.3 units/ml N-glycanase.

Detailed Description Text (63):
Direct confirmation of the identity of the 16 kD chymotryptic fragment of u-PAR (see "Results" below) to the binding domain of the receptor requires a cross-linking experiment using non-labelled DFP-u-PA or ATF as the ligand and analysis by SDS-PAGE and silver staining, using the metods already adopted (see Example 1). For further analyses, the fragment will be generated on a preparative scale (i.e., using purified protein in the range of 20-50 .mu.g as the starting material). The N-terminal amino acid sequence of the fragment will be obtained by the methods described in Example 1 (i.e., Tricine SDS-PAGE, electroblotting and amino acid sequencing). Identification of the fragment will subsequently be done by comparison to the amino acid sequence derived from u-PAR cDNA. For a closer identification of the binding determinant, synthetic peptides covering the chymotryptic fragment will be constructed. The peptides will be assayed for their potential inhibitory activity against the binding reaction between u-PAR and the ligand, as studied by cell binding assays (Nielsen et al., 1988; Appella et al., 1987) or by chemical cross-linking assay.

Detailed Description Text (67):
In parallel, the samples were analyzed in the chemical cross-linking assay, using .sup.125 I-ATF as the ligand (FIG. 5). While the non-degraded samples (lanes 4 and 5) showed the 70-75 kD conjugate band which is characteristic for the intact u-PAR (see Example 1), the intensity of this band was much reduced in the degraded samples (lanes 1-3). In contrast, the degraded samples showed an approx. 30 kD cross-linked conjugate; i.e. the size to be expected for a conjugate formed between the above mentioned, 16 kD u-PAR degradation product and the 15 kD ATF. The presence of a minor binding activity corresponding to intact u-PAR was ascribed to the cleavage being slightly incomplete; compare to the molecular weight pattern of FIG. 4. When analysis was preceeded by phase separation in the Triton X-114 system, it came out that th 30 kD conjugate was formed by a product preferentially present in the buffer phase, whereas the binding activity corresponding to intact u-PAR partitioned into the detergent phase (not shown).

Detailed Description Text (69):
In conclusion, the only detectable u-PAR fragment in the lower molecular weight (i.e., below 40 kD) region, formed by chymotrypsin in the concentration range tested, was a 16 kD product, consistent with the expected size for the fragment with binding activity observed after cross-linking to .sup.125 I-ATF. Unlike the intact u-PAR, the ligand binding fragment proved hydrophilic in the Triton X-114 system,

suggesting that this fragment does not include the diacylglycerol part of the protein (see Example 4). The deglycosylation experiment showed that the ligand binding fragment is glycosylated and suggested that the polypeptide part of the fragment comprised only 6-10 kD, corresponding to approx. 50-90 amino acid residues.

Detailed Description Text (76):
The library was screened with synthetic oligonucleotide probes made on the basis of amino acid sequence data from purified receptor protein (Tables 4-5). The melting temperatures were calculated from Lathe, J. Mol. Biol. 183: 1-12, 1985. The equation used was modified from:

Detailed Description Text (79):
The hybridization conditions were then further tested in pilot experiments to maximize the signal to noise ratio. Briefly, nitrocellulose filters containing DNA from the plasmid library were hybridized to the end-labelled oligonucleotide probe at various temperatures and salt concentrations (all within the range calculated from Lathe, supra). The filters were produced according to Grunstein and Hogness ("Colony hybridization: A method for the isolation of cloned DNAs that contain a specific gene", Proc. Natl. Acad. Sci. USA 72: 3961, 1975). The hybridization conditions to be used for the screening were chosen as the ones giving the minimum amount of background hybridization. In Table 3, the amino acid sequence derived frm a preliminary amino-terminal sequencing of purified u-PAR (see Example 1) and the derived oligonucleotide sequence are presented.

Detailed Description Text (81):
Initially, the plasmid library was screened with the N-terminal probe using the procedure of Grunstein and Hogness (supra). The detailed procedure is described below. Several positive clones were found but after the third rescreening, only one remained. The purity of the clone was checked and DNA was prepared from it (see large scale DNA preparation below). The DNA was digested with several different restrictions enzymes, and a map of the restriction sites found in the clone was constructed (see procedure in Maniatis et al., Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, 1982). The insert was further analysed by DNA sequencing (see procedure below). The clone was able to code for 7 out of the 8 amino acids in the N-terminal peptide used to construct the 24-mer probe. The sequence in the probe starts with an A whereas the clone had a T in this position, resulting in the substitution of Cys for Met. The clone was thus isolated by a specific hybridization but could not code for the correct peptide.

Detailed Description Text (99):
The complete sequence of one of the isolated clones (p-u-PAR-1) was obtained on double-stranded DNA in both orientations using commercial primers for pEMBL18 (M13 primers) and internal synthetic primers (see above). The sequence is shown in Sequence (1) in the Detailed Description of the invention. The restriction map and the sequencing strategy are illustrated in FIGS. 7A-C. The cDNA clone is 1364 nucleotides long from the 5' end to the beginning of the polyA stretch. At the 5' end, 46 nucleotides precede the first ATG codon which is followed by a 1005 nucleotides sequence with an open reading frame, ending with a nonanucleotide containing two in frame stop codons. 312 nucleotides of 3' untranslated sequence separate the first stop (TAA) codon from the polyA sequence. The assignment of the ATG at nucleotide 47 as the translation start site agrees with the consensus for initiating regions (Kozak, 1987) as discussed above. The translated sequence starts with a hydrophobic sequence which conforms to the rules for the signal peptide (von Heijne, 1986) (see above). The putative signal peptide is followed by 313 amino acid residues. The sequence shown in Sequence (1) was compared with the initial amino terminal sequence (FIG. 7A), and it was observed that in fact the original sequence contained an error at position 6 (Asn instead of Cys) which, however, did not prevent the isolation of the right cDNA clone. This is in fact proven by the 25/26 matches of the sequence derived from the cDNA with the definitive N-terminal protein sequence (see Example 1) determined in the course of this study after carboxymethylation and electroblotting of the purified protein [the region of homology is underlined in sequence (1)]. The calculated amino acid content agrees well with the one measured on the U937 protein (see Example 1). Also the calculated molecular weight (34,633) agrees well with the migration of the deglycosylated

protein (see Example 1).

Detailed Description Text (100):
The human u-PAR is a relatively small protein of 313 amino acid residues. The amino acid sequence contains five potential N-linked glycosylation sites, in agreement with the high level of glycosylation of the protein (see Example 1). Starting at amino acid position 282, a sequence of 21 hydrophobic amino acids flanked by arginine residues may represent a membrane spanning domain of the u-PAR (FIG. 7C). At the C-terminal (possibly intracellular) side of the presumptive membrane-spanning segment, the arginine is followed by 9 additional hydrophobic amino acids ending with a carboxy-terminal threonine. Because of the high hydrophobicity of the ten carboxy-terminal residues, u-PAR may contain no intracytoplasmic domain at all, i.e. also the carboxy-terminal 10 residues may be buried in the membrane. The sequence of the carboxy-terminal about 30 amino acid residues would also be compatible with a signal peptide for glycolipid-anchored, phospholipase C-sensitive membrane attachment (Ferguson and Williams, 1988). The u-PAR is a slightly acidic protein (6 net acid charges), is very rich in cysteine, rich in glycine and leucine, and poor in lysine. The u-PAR is also rich in serine and threonine residues, which might indicate O-linked glycosylation (Russell et al., 1984). However, deglycosylation and sugar composition studies indicate that the receptor contains only N-linked carbohydrates (see Example 1).

Detailed Description Text (102):
Further studies of the u-PAR amino acid sequence revealed that the entire extracellular portion of the molecule is organized into three homologous cysteine rich domains (1-92, 93-191, and 192-281) as follows: ##STR5## (Amino acid residues that are identical in at least two of the repeats are indicated through underlining and italics while conservative substitutions are indicated with italics only).

Detailed Description Text (128):
Receptors are anchored at the plasma membrane by a stretch of hydrophobic amino acids (the trans-membrane domain) or through a glycolipid anchor. Most integral membrane proteins have a single trans-membrane domain, although cases have been described of multiple trans-membrane domains. In many cases, the trans-membrane domain is present in the middle of the protein sequence, i.e. between the carboxy terminal portion (generally intracellular) and the amino terminus (generally extracellular, containing the binding site for the ligand in the case of most receptors). A carboxy-terminal hydrophobic region is also a signal for glycolipid-anchor processing.

Detailed Description Text (129):
The available information on the structure of the U-PAR indicates that it is a protein of about 35,000 daltons, i.e. about 330 amino acids.

Detailed Description Text (130):
An amino acid sequence compatible with both a trans-membrane domain and a glycolipid anchor signal is present at the carboxy terminus.

Detailed Description Text (131):
In order to produce a soluble receptor, it is necessary to modify the protein in such a way as to eliminate the hydrophobic, membrane-spanning domain or the glycolipid anchor signal, while retaining both the signal sequence for secretion and the extracellular, ligand-binding portion of the u-PAR. To this end, two constructions have been made. In one of these, the carboxy-terminal 8 last amino acids have been eliminated by inserting a stop codon at the unique PFLM-1 site of the u-PAR cDNA. The following sequence depicts the carboxy-terminal region of the normal u-PAR:

Detailed Description Text (134):
which codes for a u-PAR molecule ending with Arg Leu and thus missing the last 8 amino acids (mutant p-u-PAR-PFLM-1). This clone has been deposited as plasmid DNA in the Deutsche Sammlung von Mikroorganismen, Mascheroder Weg 1b, D-3300 Braunschweig, Federal Republic of Germany, on 27 Mar. 1990, in accordance with the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, and has received Accession No.

DSM 5865.

Detailed Description Text (135):
p-u-PAR-PFLM-1 clone has been transfected into LB6 cells as described above and its expression compared with that of wildtype p-u-PAR-1 cDNA. As shown in FIG. 11, this mutant expresses a u-PAR molecule that is partly recovered in the medium and partly retained in the cells. In fact, cross-linking to iodinated ATF shows a single band in the medium and two bands in the Triton X-114 extract (prepared as described in Example 1). The lower molecular weight band corresponds to a molecular weight of the non-glycosylated u-PAR. Only the high molecular weight band is present on the cell surface (see below). The data presented in FIG. 11 indicate that approximately 10 times as much protein is present in the medium with respect to what is retained in the cell.

Detailed Description Text (136):
A second mutant has been prepared in which the carboxy-terminal 36 amino acids have been deleted from the u-PA receptor, thus leaving a protein with no trans-membrane and no glycolipid anchor domain. To obtain this mutant, oligonucleotide-directed mutagenesis was employed, using the system commercially available from Amersham, to insert a single EcoRV site. To this end, the following oligonucleotide was used which hybridizes to the nucleotides 935-952 of the u-PAR cDNA sequence:

Detailed Description Text (144):
Acetylcholinesterases from human and bovine erythrocytes, phospholipase A.sub.2 from bee venom and myelin basic protein from bovine brain were from Sigma. Phospholipase D from cabbage and phosphatidylinositol-specific phospholipase C from Bacillus cereus (PI-PLC) were from Boehringer Mannheim. u-PAR was purified from PMA-stimulated U937 cells as in Example 1. Active human u-PA was purchaged from Serono and was DFP-inactivated as described (Nielsen et al., 1988); the amino terminal fragment (ATF) of u-PA was a kind gift from Dr. G. Cassani (LePetit, Italy). ATF, u-PAR and DFP-inhibited u-PA were radiolabelled as described (Nielsen et al., 1988) except that 0.1% (v/v) Triton X-100 was replaced by 0.1% (w/v) CHAPS in the case of u-PAR and by 0,01% (v/v) Tween 80 in the case of ATF and DFP-u-PA. Preparation of polyclonal rabbit antibodies against human u-PAR was carried out as described in Example 11.

Detailed Description Text (148):
Cell culture was performed as described in Example 1. Prior to metabolic labelling human U937 cells (5.times.10.sup.7 cells/dish) were PMA-stimulated (150 nM) for 5 hours in order to increase expression of u-PAR. For labelling with [.sup.3 H]ethanolamine and [.sup.3 H]myristic acid the cells were cultured in RPMI 1640 medium, while labelling with myo-[.sup.3 H]inositol was performed in Eagle's minimum essential medium. Both media were supplemented with: 2 mM L-glutamine, 5 mM Na-pyruvate, 200 units/ml penicillin, 25 .mu.g/ml streptomycin, 25 mM HEPES (pH 7.4), 0.5 mg/ml defatted BSA and 4.times.normal concentration of non-essential amino acids. All tracers were added from stock solutions in 25 mg/ml defatted BSA, 0.1M HEPES (pH 7.4) to a final concentration of 0.1 mCi/ml in 10 ml media and metabolic labelling was allowed to proceed for 15 hours at 37.degree. C. Subsequently, the adherent cells were acid treated, washed and lyzed with 5 ml ice-cold 1% precondensed Triton X-114, 0.1M Tris (pH 8.1), 10 .mu.g/ml Trasylol, 1 mM PMSF and 0,2 mM ZnCl.sub.2. Finally, detergent-phase separation was performed as described in Example 1.

Detailed Description Text (151):
Tricine-SDS-PAGE and amino acid analysis

Detailed Description Text (153):
The Coomassie stained u-PAR was prepared for amino acid analysis by acid hydrolysis directly on the excised PVDF-membrane at 110.degree. C. in 100 .mu.l of redistilled 6M HCl including 0.05% (w/v) phenol and 5 .mu.l of 1% (w/v) DTDPA in 2M NaOH as published (Ploug et al., 1989). Amino acid analysis was performed on a Waters amino acid analyzer, equipped with o-phtaldialdehyde derivatization essentially as described (Barkholt and Jensen, 1989). However, the chromatographic system was modified slightly to increase resolution of basic amino acids. Elution was still performed by a pH-gradient resulting from mixing two non-halide buffers A and B (for

composition see Barkholt and Jensen, 1989), but the gradient consisted of the following linear segments: initial eluant 100% A, 88% A and 12% B at 15 min, 60% A and 40% B at 24 min, 55% A and 45% B at 26 min, 50% A and 50% B at 36 min, 30% A and 70 B at 40 min, 25% A and 75% B at 64 min, 100% A at 65 min and 100% A from 65 to 70 min.

Detailed Description Text (155):
SDS-PAGE, chemical cross-linking with disuccinimyl suberate (DSS) and an analytical detergent phase separation was performed with Triton X-114 as described in Example 1.

Detailed Description Text (158):
Amino acid analysis of purified u-PAR

Detailed Description Text (159):
Amino acid analysis of the purified u-PAR (see Example 1) revealed the presence of an unidentified compound in the acid hydrolysate that reacted with o-phtaldialdehyde and eluted just after ammonia during cation-exhange chromatography (FIG. 12). A similar peak was observed when u-PAR was purified from non-stimulated U937 cells (2.times.10.sup.10 cells), but otherwise treated identically (data not shown). This unknown compound behaved as a covalent constituent of u-PAR, as it persisted within the purified protein despite boiling it in 2% SDS followed by Tricine-SDS-PAGE and electroblotting onto a 0.45 .mu.m polyvinylidene diflouride (PVDF) membrane in the presence of 10% (v/v) MeOH. Furthermore, the compound was a specific constituent of the Coomassie stained u-PAR, as it was absent, when appropriate pieces of PVDF-membranes just above and below the protein stained area were excised and prepared for amino acid analysis by the same procedure (FIG. 12 insert). In addition, several stained proteins and peptides previously analyzed by this approach did not reveal the presence of this particular component (Ploug et al., 1989).

Detailed Description Text (160):
For amino acid analysis in this study, a special gradient was designed for the cation-exchange chromatography that allowed an increased resolution of common as well as various uncommon, basic amino acids without impairing reproducibility of their retention times (see Materials and Methods section). By this method the unidentified compound in u-PAR reproducibly eluted after 55.3 min, between ammonia (53.5 min) and arginine (60.8 min). As various physiological occurring arginine derivatives are expected to possess approx. similar retention times, several methylated arginine derivatives were tested, including: N.sup.w,N.sup.w -dimethylarginine (53.8 min), N.sup.w,N.sup.w -dimethylarginine (54.4 min) and N.sup.w -monomethylarginine (58.6 min). None of these retention times were in agreement with the one observed for the unidentified compound in u-PAR. However, when authentic ethanolamine was tested, it showed exactly the same retention time as that for the unidentified compound. Furthermore, upon hydrolysis of both human and bovine erythrocyte acetylcholinesterases, a compound with this retention time was also observed, whereas it was absent in the hydrolysate from e.g. myelin basic protein. Acetylcholinesterases isolated from erythrocytes contain ethanolamine as a covalent constituent in a glycolipid membrane anchor, while myelin basic protein posseses a partly methylated arginine residue. It is therefore concluded that u-PAR does contain ethanolamine, covalently linked to the protein by acid labile bonds (e.g. ester or amide bonds). Quantitative analysis of the data in FIG. 12 shows that each u-PAR molecule contains 2-3 ethanolamine residues (see also Table 5).

Detailed Description Text (167):
When purified u-PAR was subjected to detergent-phase separation by Triton X-114, it almost quantitatively partitioned into the detergent phase, as assessed by cross-linking to .sup.125 I-labelled ATF (FIG. 15A), thus demonstrating the very hydrophobic properties of the receptor. Incubation with PI-PLC altered the hydrophobicity of the u-PA binding protein substantially, as more than 50% of the ATF-binding activity was now recovered in the aqueous phase (FIG. 15B). It proved impossible to achieve a higher level of this conversion in the purified u-PAR preparation by increasing the concentration of PI-PLC. These data are in accordance with the fraction of cell associated u-PA which had been released in the previous experiment by PI-PLC treatment of intact PMA-stimulated U937 cells (FIG. 13). This finding may indicate that a partial resistance (approx. 50%) against bacterial

PI-PLC is a genuine feature of the u-PAR population In vivo. Other phospholipases (PLD and PLA.sub.2) did not induce any significant change in the hydrophobic properties of the purified u-PAR (FIG. 15C).

Detailed Description Text (172):
Apart from demonstrating the presence of approx. 2 mol ethanolamine/mol u-PAR (FIG. 12 and Table 5), amino acid analysis revealed additional information about potential post-translational processing of this membrane receptor. When the calculated amino acid composition for the purified u-PAR was compared with that predicted for the nascent protein from cDNA sequence, several reproducible and significant discrepancies arose (Table 5). In particular, the actual determinations of Ala and Leu were too low, whereas those of Tyr and Phe were too high (Table 5). Interestingly, however, it was possible to bring the calculated and the predicted amino acid compositions into perfect agreement provided that the last 29-31 COOH-terminal residues were removed during some posttranslational event (Table 5). Thus, on the basis of the determined amino acid composition and the accuracy/precision normally obtained for this equipment, it is assumed that there exists a COOH-terminal processing site in u-PAR. According to this model, processing is expected to occur at one of the residues Ser.sub.282, Gly.sub.283 or Ala.sub.284 --as indicated in FIG. 16.

Detailed Description Text (190):
The effect of PMA on production of u-PAR protein was studied by cross-linking experiment. .sup.125 I-labelled aminoterminal fragment (ATF) of the urokinase were chemically cross linked to the detergent phase of phase-separated Triton X-114 extracts prepared from U937 cells treated with PMA for different time periods. FIG. 18 shows a weak signal of .sup.125 I-ATF cross-linked to the u-PAR in control U937 cells. After increasing time of PMA treatment both an increase in the strength of signal and a change to a lower electrophoretic mobility was seen.

Detailed Description Text (212):
Human fibrosarcoma cells (HT-1080, CCL 121) were obtained from the American Type Culture Collection, Rockville, Md. Confluent cell layers were grown in plastic Linbro wells (2 cm.sup.2 ; Flow Laboratories) in Eagle's minimal essential medium (MEM) supplemented with 10% heat-inactivated (56.degree. C. for 60 minutes) fetal calf serum (Gibco), 100 IU/ml penicillin and 50 .mu.g/ml streptomycin. After reaching confluence, the cells were rinsed three times with MEM containing 0.2% bovine serum albumin (BSA), then changed to either serum-free medium (0.5 ml) or medium containing 10% heat-inactivated and plasminogen-depleted (i.e. absorbed with lysine-Sepharose; Pharmacia, Uppsala, Sweden) fetal calf serum as indicated in the Examples.

Detailed Description Text (214):
Human plasminogen (with glutamic acid N-terminal) was prepared by affinity chromatography on lysine-Sepharose (Deutsch, D. G., and E. T. Mertz, "Plasminogen: Purification from human plasma by affinity chromatography", Science 170: 1095-1097, 1970) from freshly separated, unfrozen human plasma pretreated with 10 .mu.M p-nitrophenyl guanidinobenzoate, 1 mM phenylmethylsulfonylfluoride and 0.1 .mu.g/ml of an anti-catalytic murine monoclonal IgG antibody to human t-PA (ESP-2; see MacGregor, I. R. et al., "Characterization of epitopes on human tissue plasminogen activator recognised by a group of monoclonal antibodies", Thromb. Haem. 53: 45-50, 1985); American Diagnostica, Greenwich, Conn.).

Detailed Description Text (215):
Inhibition studies made use of the following reagents added to cell cultures: an anti-catalytic murine monoclonal IgG antibody to human plasmin (anti-plg 1, 20 .mu.g/ml; see Sim, P-S. et al., "Monoclonal antibodies inhibitory to human plasmin: definitive demonstration of a role for plasmin in activating the proenzyme of urokinase-type plasminogen activator", Eur. J. Biochem. 158: 537-542, 1986); aprotinin (Trasylol, Bayer, Leverkusen, FRG; 200 KIU/ml); tranexamic acid (Cyclokapron, Kabi Vitrum, Stockholm; 10 .mu.M and 100 .mu.M); human type-2 plasminogen activator inhibitor minactivin (see Golder, J. P. et al., "Minactivin: A human monocyte product which specifically inactivates urokinase-type plasminogen activators", Eur. J. Biochem. 136: 517-522, 1983), PAI-2 purified from cultures of human U-937 histiocytic lymphoma cells (see Leung, K-C. et al., "The resistance of

fibrin-stimulated tissue plasminogen activator to inactivation by a class PAI-2 inhibitor (minactivin)", Thromb. Res. 46: 755-766, 1987) titration equivalent of 3.6 IU u-PA/ml; an anti-catalytic murine monoclonal IgG antibody to human u-PA (clone 2 (10 .mu.g/ml) in Nielsen, L. S. et al., "Enzyme-linked immunosorbent assay for human urokinase-type plasminogen activators and its proenzyme using a combination of monoclonal and polyclonal antibodies", J. Immunoassay 7: 209-228, 1986); the anti-catalytic monoclonal antibody to human t-PA (10 .mu.g/ml); a neutralising murine monoclonal IgG antibody to human PAI-1 (Nielsen, L. S. et al., "Monoclonal antibodies to human 54,000 molecular weight plasminogen activator inhibitor from fibrosarcoma cells--inhibitor neutralization and one-step affinity purification", Thromb. Haem. 55: 206-212, 1986) (10 .mu.g/ml) and diisopropyl fluorophosphate (DFP)-inactivated u-PA (0-10 .mu.g/ml).

Detailed Description Text (217):
Active two-chain u-PA (Ukidan, Serono) was dissolved in 0.1M Tris-HCl, pH 8.1, 0.1% Tween 80 (Tris/Tween). A freshly prepared solution of 500 mM DFP (Sigma) in isopropanol was added to yield a final DFP concentration of 5 mM. After thorough mixing, the sample was incubated for 2 hours at 37.degree. C., after which period addition of DFP was repeated as above. After renewed incubation for 2 hours at 37.degree. C., the reaction was terminated by thorough dialysis at 0.degree. C. against Tris/Tween. No residual DFP inhibitor could be detected when the preparation was tested in an activity assay of soluble urokinase.

Detailed Description Text (221):
Cell culture supernatants were assayed for pro-u-PA and active u-PA by the following modification of an immunocapture method (Stephens et al., 1988; Stephens et al., 1987). Microtitre wells of polystyrene immunoplates (type 269620, A/S Nunc, Roskilde, Denmark) were coated overnight at 37.degree. C. with 50 .mu.l of a solution of goat IgG antibodies to human u-PA (cat. #398, American Diagnostica). The coating solution contained 2.5 .mu.g of IgG per ml of 0.1M sodium carbonate (pH 9.8). After rinsing, the wells were treated with conditioned medium (50 ml) for 2 hours at 23.degree. C., then rinsed again. Half the wells were then treated with 50 .mu.l of freshly prepared 2 .mu.M p-nitrophenyl guanidinobenzoate (NPGB, Sigma) (Dan.o slashed., K., and E. Reich, "Plasminogen activator from cells transformed by an oncogenic virus--Inhibitors of the activator reaction", Biochim. Biophys. Acta 566: 138-151, 1979) for 20 minutes at 37.degree. C. The other half (controls) received 50 .mu.l of rinsing buffer (0.05% Tween 20 in PBS). After rinsing, u-PA was assayed in all the wells by addition of 40 .mu.l of plasminogen solution (100 .mu.g/ml in assay buffer consisting of 50 mM sodium glycinate (pH 7.8), 0.1% Triton X-100, 0.1% gelatin and 10 mM 6-aminocaproic acid which also contained a very low concentration of plasmin (10 ng/ml)), and incubation took place for 30 minutes at 37.degree. C. This concentration of plasmin in the plasminogen incubation was sufficient to enable full realization of the potential activity of pro-u-PA (cf. Petersen et al., 1988). The plasmin produced by this incubation was assayed by its thioesterase activity (Green, G. D. G., and E. Shaw, "Thiobenzyl benzyloxycarbonyl-L-lysinate, substrate for a sensitive calorimetric assay for trypsin-like enzymes", Anal. Biochem. 93: 223-226, 1979) by the addition of 200 .mu.l of a solution containing 200 mM potassium phosphate (pH 7.5), 200 mM KCl, 0.1% Triton X-100, 220 .mu.M Z-lysine thiobenzyl ester (Peninsula Laboratories, Belmont, Calif.) and 220 .mu.M 5,5'-dithiobis(2-nitrobenzoic acid) (Sigma). This mixture was incubated for 30 minutes at 37.degree. C., and the absorbancies of the wells were read at 405 nm. Active u-PA (60,000 IU/mg) was purchased from Calbiochem-Behring (La Jolla, Calif.) and pro-u-PA (potential activity 90,000 IU/mg) was obtained from American Diagnostica.

Detailed Description Text (222):
Pro-u-PA and active u-PA bound to the cell layer were recovered for immunocapture assays by the same method as was used in the metabolic labelling (see above). Each culture well (2 cm.sup.2) was eluted with 150 .mu.l of acid glycine at pH 3 (Stoppelli et al., 1986). For conditioned medium and cell-bound u-PA, the u-PA activity assayed after NPGB treatment was expressed as a percentage of the total activity obtained without NPGB treatment, and this percentage used as an index of pro-u-PA content (pro-u-PA index). The conditions used for the NPGB treatment were previously established (Stephens et al., 1988) to allow selective inactivation of active u-PA, while leaving the pro-u-PA unchanged and still able to be activated by

the added plasmin to the same extent as untreated pro-u-PA.

Detailed Description Text (225):
Plasmin bound to the cell layer was recovered and assayed as follows. After harvest of culture medium, the cells were rinsed three times with PBS (plasmin assays of further rinses were negative); then the bound plasmin was specifically eluted (Miles, L. A., and E. F. Plow, "Binding and activation of plasminogen on the platelet surface", J. Biol. Chem. 260: 4303-4311, 1985) with a solution of 1 mM tranexamic acid in the same rinsing solution (150 .mu.l/well). Plasmin activity was assayed in eluate samples (50 .mu.l) as above with an incubation time of 3 hours at 37.degree. C. Tranexamic acid at 1 mM had no effect on the thioesterase activity of plasmin in these assays.

Detailed Description Text (228):
After addition of purified preparations of human plasminogen to cultures of human fibrosarcoma cells (HT-1080) growing in a medium with 10% plasminogen-depleted fetal calf serum, plasmin activity could be recovered as a bound fraction from the cell layer. Upon varying the concentration of added plasminogen, the bound plasmin activity increased in a dose-dependent manner (FIG. 22). The binding was specific so that after rinsing of the cells with isotonic buffer, the plasmin could be released by 1 mM tranexamic acid. This agent disrupts interactions with plasminogen or plasmin which involve the lysine affinity sites of the heavy-chain kringles (Miles, supra). The plasmin released from HT-1080 cell surfaces was conveniently measured by its thioesterase activity, a method which was unaffected by the presence of tranexamic acid. Some plasmin activity was also detected in the medium. At a concentration of 40 .mu.g/ml human plasminogen added to 0.5 ml of medium above a confluent 2 cm.sup.2 cell layer, activity corresponding to 28 ng of plasmin could be recovered from the cell layer with tranexamic acid, while 10 ng was measurable in the medium after 3 hours of incubation at 37.degree. C. This concentration of plasminogen is well below the 200 .mu.g/ml present in normal human plasma.

Detailed Description Text (231):
Incubation of cells carrying plasmin with fresh serum-free medium showed that approximately 40% of the activity remained bound after 2 hours at 37.degree. C. (FIGS. 24A and 24B). When the cells were incubated in 10% serum-containing medium, the same fraction (40%) of this activity could be recovered from the cells; the bound plasmin was not inactivated by the serum. However, only about 11% (compared to 60% for serum-free medium) could be detected in the serum-containing medium (FIG. 24B). When 1 mM tranexamic acid was added to the serum-containing medium, no plasmin activity could be recovered from the cells (FIG. 24A).

Detailed Description Text (241):
To prevent the interference of PAI-1, the neutralizing PAI-1 antibody was therefore included in the next experiment in which the effect of the plasmin inhibitor aprotinin and the effect of an anti-catalytic monoclonal antibody to human plasmin on the conversion of pro-u-PA to active u-PA were studied. As shown in Table 7, both these inhibitors increased the relative amount of pro-u-PA, thus demonstrating that the activation of cell-bound pro-u-PA was catalyzed by plasmin. To study whether this was an effect of cell-bound plasmin, the effect of tranexamic acid in a concentration of 100 .mu.M was also tested, which concentration completely inhibits binding of plasmin to the cells, but does not affect the ability of plasmin to activate pro-u-PA in solution (R. Stephens, unpublished results). This treatment markedly decreased the relative amount of active u-PA, indicating that the activation of the cell surface pro-u-PA is catalyzed by the surface-bound plasmin.

Detailed Description Text (242):
The following additions were made to cell layers growing in MEM medium (0.5 ml) containing 10% heat-inactivated and plasminogen-depleted fetal calf serum: native human plasminogen (Plg, 40 .mu.g/ml); anti-catalytic monoclonal antibody to human u-PA (10 .mu.g/ml); anti-catalytic monoclonal antibody to human t-PA (10 .mu.g/ml); PAI-2 (titration equivalent of 3.6 UI u-PA/ml); anti-catalytic monoclonal antibody to human plasmin (20 .mu.g/ml); aprotinin (200 KIU/ml); tranexamic acid (TA, as shown). The cultures were incubated for the times shown before assay of cell-bound plasmin. The incubation with plasminogen was used as the 100% control for bound plasmin.

Detailed Description Text (244):
Confluent cell layers were incubated for 2 hours at 37.degree. C. with MEM medium (0.5 ml) containing 10% heat-inactivated and plasminogen-depleted fetal calf serum with the following additions: native human plasminogen (Plg, 40 .mu.g/ml); neutralizing monoclonal antibody to human PAI-1 (10 .mu.g/ml); aprotinin (200 KIU/ml); anti-catalytic monoclonal antibody to human plasmin (20 .mu.g/ml); and tranexamic acid (TA, as shown). Half the wells were then treated with aprotinin (200 KIU/ml) and used for assay of bound u-PA and its pro-u-PA index. The other half were used for elution and assay of bound plasmin.

Detailed Description Text (252):
Binding assay. Before binding, U937 cells were incubated for 1 hour at 4.degree. C. in RPMI 1640 medium supplemented with 0.1% bovine serum albumin and 50 mM Hepes (pH 7.4). The cells were then acid-treated in 50 mM glycine-HCl, 100 mM NaCl (pH 3) for 3 minutes at 4.degree. C. and quickly neutralized with half a volume of 0.5M Hepes, 100 mM NaCl (pH 7.4). One million cells were then resuspended in 0.2 ml of binding buffer (phosphate buffered saline supplemented with 0.1% bovine serum albumin) containing iodinated ligands (about 50,000 cpm corresponding to 0.1 nM for ATF and 0.05 nM for pro-u-PA and u-PA) and incubated for the indicated time at 4.degree. C. After binding, the cells were centrifugated and washed with cold phosphate buffered saline--0.1% bovine serum albumin. Non-specific binding was determined in the presence of 100 nM unlabelled u-PA.

Detailed Description Text (254):
Amidolytic assay. u-PA activity was assayed by incubating 100 .mu.l aliquots of binding mixtures or supernatants of binding assays in 0.05M Tris-HCl (pH 7.5), 40 mM NaCl, 0.01% Tween 80, with 1 mM of the plasmin-specific substrate S2390 (Kabi Vitrum, Sweden) and 0.5 .mu.M plasminogen in a final volume of 0.3 ml. The time dependence of the colour development was measured following the absorbance at 405 nm (Petersen et al., 1988).

Detailed Description Text (266):
Surprisingly, in the absence of PAI-1 in the binding mixture, two weaker bands with molecular -weights of about 69 and 90 kD are detected. This background was dependent on the presence of the cells and could not be eliminated by different pretreatment of the cells. These bands were not retained on Sepharose 4B columns coupled with anti-PAI-1 IgG. This is in contrast to the complexes found on cells after incubation with preformed PAI-1/u-PA complexes which, as expected, could be isolated from the acid washes of cells by immunoaffinity chromatography (data not shown). This is in agreement with the very low levels of PAI-1 in U937 cells (Lund et al., 1988). The nature of the two contaminating bands, therefore, remains unknown and will require further investigation. They may represent complexes of receptor-bound u-PA with PAI-2 (Genton et al., 1987) or with protease nexin-1 (Baker et al., Cell 21: 37-47, 1980).

Detailed Description Text (282):
Unlike the internalization of the nexin-protease complexes which are formed in solution and subsequently bind to the cells and are internalized via so far uncharacterized receptors (Baker et al., 1980), the u-PA:PAI-1 complex is bound to the receptor itself (see Example 8) and subsequently undergoes internalization and degradation. This receptor, therefore, must alternate between two possible configurations: one in which it binds active u-PA and in which it dictates plasminogen activation on the cell surface; and another in which it binds the inhibited enzyme and in which it favours internalization and degradation of the ligand. This property could be exploited for internalizing toxins and thus specifically kill the cells that express the u-PA receptor, or by forcing the state of the receptor from one state (i.e. exposed) to another, through PAI-1 or PAI-1 analogues.

Detailed Description Text (286):
Plasminogen was purified from fresh human plasma as previously described (Dan.o slashed. and Reich, 1979), and was further separated into its two isoforms by elution from lysine-Sepharose with a linear gradient of 6-amino-hexanoic acid. Plasminogen isoform 2 was used in all experiments described here. u-PA (M.sub.r

55,000) was obtained either by plasmin activation of pro-uPA (Ellis et al., 1987) or as Ukidan (Serono). Both preparations were greater than 95% high molecular weight u-PA by SDS-polyacrylamide gel electrophoresis. The concentration of active u-PA in these preparations was determined by active-site titration with p-nitrophenyl-p-guanidinobenzoate (Sigma Chem. Co.). DFP-inactivated u-PA was prepared as described in Example 1. The murine monoclonal antibody to u-PA was clone 2 from Nielsen et al., 1986. Active PAI-1 was purified from the serum-free conditioned medium of Hep G2 cells by affinity chromatography on immobilized anhydro-urokinase (Wun et al., 1989). PAI-2 was purified from U937 cell lysates by chromatofocusing as described (Kruithof et al., 1986). The concentrations of active inhibitor in the various PAI preparations were determined by titration against u-PA immediately before use in the kinetic experiments. PAI-1 or PAI-2 at varying concentrations between 1 nM and 100 nM were incubated with active-site titrated u-PA (20 nM) for 1 hour at 37.degree. C. in 0,05M Tris, 0,1M NaCl pH 7,4 containing 0,2% bovine serum albumin. Residual u-PA activity was then measured by hydrolysis of 0,2 mM Glu-Gly-Arg-AMC (Bachem, Switzerland).

Detailed Description Text (347):
2) Reaction buffer: 0.1% bovine serum albumin+0.1% Triton X-100 in PBS (0.1% BSA, 0.1% Triton X-100/PBS).

Detailed Description Text (368):
6) Washing buffer: PBS+0.1% Tween 20, pH 7.4 (PBS/Tween 20).

Detailed Description Text (390):
5) PBS+0.1% Tween 20, pH 7.4 (PBS/Tween 20).

Detailed Description Text (412):
Samples of purified human u-PA receptor (Example 1) were subjected to SDS-polyacrylamide gel electrophoresis under non-reducing conditions on a 6-16% gradient gel. By the use of fluorescent molecular weight markers run in neighbouring lanes, the electrophoretic region corresponding to the antigen was excised. The gel piece was lyophilized and subsequently macerated in a Mikro-Dismembrator II apparatus (B. Braun AG, Federal Republic of Germany). The polyacrylamide powder was reconstituted in Tris-buffered saline, mixed with Freund's incomplete adjuvant and used for injection of a New Zealand white rabbit. The animal received 5 injections, each containing approximately 3 .mu.g of the antigen, over a 10 week period, followed by a single 8 .mu.g injection after an additional 7 weeks. Serum was drawn 1 week after the last injection, and IgG was prepared by Protein A-Sepharose chromatography. In order to remove antibodies against trace impurities in the injected antigen, the antibody was absorbed by consecutive passages through columns containing immobilized human u-PA and the protein mixture constituting the Triton X-114 detergent phase from PMA-stimulated U937 cells (see Example 1), respectively. The antibody preparation obtained did not inhibit the amidolytic or plasminogen activator activity of u-PA in solution.

Detailed Description Text (415):
Western Blotting--Samples of affinity purified u-PAR or detergent phase from Triton X-114 extracts of PMA-stimulated U937 cells were subjected to SDS-PAGE under reducing conditions on 6-16% gradient gels. The gels were electroblotted onto nitrocellulose sheets. The sheets were rinsed and blocked with 30% fetal calf serum in Tris-buffered saline, pH 7.4. The sheets were incubated with mouse anti-u-PAR serum or control serum (i.e. mouse antiserum against porcine mucins), diluted in fetal calf serum in Tris-buffered saline. The sheets were rinsed, incubated with secondary antibody (alkaline phosphatase-conjugated rabbit anti-mouse Ig (Dakopatts, Copenhagen)), and developed with nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate/Levamisol.

Detailed Description Text (423):
Rabbit polyclonal antibodies were prepared by immunizing a rabbit with polyacrylamide gel material containing affinity-purified u-PAR that had subsequently been subjected to preparative SDS-PAGE. The IgG fraction was isolated from the obtained antiserum and absorbed by passage through columns with immobilized human u-PA and immobilized membrane-protein mixture derived from PHA-stimulated U937 cells, respectively. The antibody recognized u-PAR in the Triton X-114 detergent

phase from PMA-stimulated U937 cells (FIG. 46A). Thus, a protein in the 50-65 kD range was recognized (lanes 1 and 2) which could be identified as being u-PAR by the ability to form a 100-110 kD conjugate with DFP-treated u-PA after the performance of chemical cross-linking (see Example 1 for methods) (lane 3). No staining was obtained with DFP-treated u-PA alone (lanes 5 and 6), and the cross-linking procedure did not alter the electrophoretic appearance of u-PAR when no DFP-treated u-PA was added (lane 2). In none of the samples was any band stained with the pre-immune IgG from the same rabbit, prepared in the same manner (FIG. 46B).

Detailed Description Text (428):
u-PA was dialyzed overnight against 0.1M Na.sub.2 HCO.sub.3 with 0.1% Triton X-100. N-biotin-hydroxysuccinimide was dissolved in N,N-dimethylformamide (5 mM). To the u-PA preparation was added 0,1 .mu.l of this solution per .mu. of u-PA, and the reaction was allowed to run for 1 hour at room temperature. Excess labelling compound was removed by dialysis overnight against 0.1M NaHPO.sub.4, pH 8.0, with 0.5M NaCl and 0.1% Triton X-100.

Detailed Description Text (429):
Cultured cells (PMA-treated U937) or cryostat sections of freshly frozen human chorion were treated for 3 minutes at room temperature with 0.05M glycine, pH 3.0 with 0.1M NaCl, neutralized with 0.5M 30 HEPES, pH 7.5 with 0.1M NaCl and incubated at 4.degree. C. with 200 nM of biotinylated DFP-treated u-PA dissolved in PBS with 0.1% BSA (PBS-BSA). Competition experiments were performed by simultaneous incubation with biotinylated DFP-treated u-PA (200 nM) and purified unlabelled u-PA (2 .mu.M).

Detailed Description Text (437):
Both assays were carried out in microtiter plates, using chromogenic substrates (see below), the cleavage of which was followed by measuring the absorbance at 405 nm in an ELISA reader. Proteolysis buffer (0.1M Tris/HCl, pH 8.1, 0.1% Triton X-100) was used as the reaction buffer and for the dilution of all samples. Affinity purified u-PAR (see Example 1) was added as indicated or substituted by a protein devoid sample of the same buffer compositon. Materials and methods not specified below were those described by Petersen et al. (1988). All samples were analysed in triplicate.

Detailed Description Text (439):
Human 54 kDa two-chain u-PA (Ukidan, Serono) was preincubated with u-PAR or buffer at the concentrations indicated for 15 min at room temperature. Plasminogen (10 .mu.g/ml final concentration) and H-D-Valyl-L-leucyl-L-lysine-p-nitroanilide dihydrochloride (Kabi product S-2251), termed substrate S1 below (final concentration 400 .mu.M) were added in a final reaction volume of 250 .mu.l, and cleavage of the substrate was followed during incubation at 37.degree. C. Standard curves were drawn from assays of the following final concentrations of u-PA: 8, 16, 32, 64, 128 and 256 pg/ml.

Detailed Description Text (441):
Human pro-u-PA was preincubated with u-PAR or buffer for 10 min at room temperature. Plasmin (10 ng/ml final concentration) was added 30 and the samples were incubated at 37.degree. C. Aliquots were taken after the following periods of incubation: 1, 2, 5, 10, 20, 30 and 60 min. After the periods indicated, plasmin activity within each sample was stopped by the addition of Trasylol (10 .mu.g/ml final concentration). Each aliquot was assayed for u-PA amidolytic activity by addition of 400 .mu.M (final concentration) of L-Pyroglutamyl-glycyl-L-arginine-p-nitronanilide hydrochloride (Kabi product (S-2444; termed substrate S2 below) in a final reaction volume of 200 .mu.l, followed by incubation at 37.degree. C. and absorbance measurement. The absorbance values were compared to a standard curve obtained with known concentrations of 54 kDa two-chain u-PA (Ukidan, Serono) in the same assay of amidolytic activity, performed simultaneously and using the same buffer composition.

Detailed Description Text (451):
Samples of purified u-PAR were treated with PI-PLC (500-fold final dilution of the Boehringer Mannheim preparation) for 30 min at 37.degree. C. This treatment led to an approx. 50% delipidation of u-PAR as judged by the shift of the ATF cross-linking activity towards the buffer phase in the Triton X114 phase separation system (see

Example 1).

Detailed Description Text (457):
Appella E, Robinson E A, Ullrich S J, Stoppelli M P, Corti A, Cassani C, Blasi F (1987) The receptor-binding sequence of urokinase. A biological function for the growth-factor module of proteases. J Biol Chem 262: 4437-4440

Detailed Description Text (462):
Barkholt V, Jensen A L (1989) Amino acid analysis: Determination of cysteine plus half-cysteine in proteins after hydrochloric acid hydrolysis with a disulfide compound as additive. Anal Biochem 177: 318-322

Detailed Description Text (469):
Bordier C (1981) Phase Separation of integral membrane proteins in Triton X-114 solution. J Biol Chem 256: 1604-1607

Detailed Description Text (485):
Eaton D L, Scott R W, Baker J B (1984) Purification of human fibroblast urokinase proenzyme and analysis of its regulation by proteases and protease nexin. J Biol Chem 259: 6241-6247

Detailed Description Text (502):
Hopp T P, Woods K R (1981) Prodiction of protein antigenic determinants from amino acid sequences. Proc. Natl. Acad. Sci. USA 78: 3824-3828

Detailed Description Text (528):
Miles L A, Plow E F (1986) Topography of the high-affinity lysine bindding site of plasminogen as defined with a specific antibody probe. Biochemistry 25: 6926-6933

Detailed Description Text (530):
Morrissey J H, Falhrai H, Edgington T S (1987) Molecular cloning of the cDNA for tissue factor, the cellular receptor for the initiation of the coagulation protease cascade. Cell 50: 129-135

Detailed Description Text (533):
Nelles L, Lijnen H R, Collen D, Holmes W E (1987) Characterization of recombinant human single chain urokinase-type plasminogen activator mutants produced by site-specific mutagenesis of lysine 158. J Biol Chem 262: 5682-5689

Detailed Description Text (544):
Ploug M, Jensen A L, Barkholt V (1989) Determination of amino acid compositions and NH2-terminal sequences of peptides electroblotted onto PVDF membranes from tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis: Application to peptide mapping of human complement component C3. Anal Biochem 181: 33-39.

Detailed Description Paragraph Table (1):
TABLE 1 Amino acid composition of affinity purified u-PAR, determined after Tricine-SDS-PAGE, electroblotting onto a PVDF membrane, and alkylation

Asp/Asn	33.2
Thr.sup.a	21.4
Ser.sup.b	26.3
Glu/Gln.sup.c	43.2
Pro	11.4
Gly	28.2
Ala	8.4
Cys (as Cys(Cm))	28.4
Val	11.9
Met.sup.d	7.7
Ile	6.7
Leu	26.5
Tyr	8.0
Phe	5.7
His	12.8
Lys	11.1
Arg	20.0
Glucosamine.sup.e	30.8

.sup.a Corrected for a 5% loss during hydrolysis. .sup.b Corrected for a 10% loss during hydrolysis. .sup.c Slight overestimation possible, due to formation of pyroglutamic acid in amino acid standard mixture. .sup.d Corrected for a 30% loss normally observed during electrophoresis and blotting (35). .sup.e Corrected for a 50% loss during hydrolysis.

Detailed Description Paragraph Table (2):
TABLE 2 N-terminal amino acid sequence of u-PAR. Parentheses indicate an identification classified as tentative. Question mark indicates no identification. Where footnotes are present, they indicate the best guess. A. Direct sequencing of affinity purified u-PAR after dialysis against 0.1M acetic acid and lyophilization. The initial yield was 70 pmol PTH-Leu at step 1. Note that direct sequencing does not allow the

identification of cysteine residues. Res. no. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Amino acid Leu ? ? Met Gln ?.sup.a Lys Thr Asn Gly Asp ? Arg Val (Glu) Glu (SEQ ID NO. 1) residue

B. Sequence obtained after Tricine-SDS-PAGE, electroblotting and alkylation. The PVDF membrane contained 35 pmol u-PAR, as estimated from a parallel amino acid analysis experiment (Table 1). The initial yield was 19.5 pmol PTH-Leu at step 1. The repetitive yield, based on Leu 1, Leu 19 and Leu 23, was 96%. Cys indicates the identification of the PTH derivative of carboxymethyl cysteine in the alkylated protein. Res. no. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Amino acid Leu ? Cys Met Gln Cys Lys Thr Asn Gly Asp Cys (Arg) Val Glu residue

Res. no. 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Amino acid Glu (His) Ala Leu Gly Gln ?.sup.b Leu ?.sup.c (Arg) Thr (Thr) Ile Val ?.sup.d (SEQ ID NO. 2) residue

.sup.a Asn .sup.b Asp .sup.c Arg/Cys .sup.d Arg/Thr

Detailed Description Paragraph Table (3):

TABLE 3 The amino acid sequence of the N-terminal peptide and the derived synthetic oligonucleotide.

##STR2## ##STR3## ##STR4## The hybridization conditions used for this probe were 5x SSC and 50.degree. C.

Detailed Description Paragraph Table (5):

TABLE 5 Amino acid composition of purified u-PAR compared with that deduced from its cDNA before and after the proposed COOH-terminal processing.sup.a Entire u-PAR sequence (Leu.sub.1 -Thr.sub.313) A) Predicted Determined after Amino acid from cDNA acid hydrolysis SD

Asp + Asn 29 32.7 0.5 Thr.sup.b 25 21.9 0.5 Ser.sup.b 25 25.8 0.5 Glu + Gln.sup.c 37 41.8 1.3 Pro 12 11.1 0.3 Gly 29 29.4 1.1 Ala 11 8.3 0.1 Cys.sup.d 28 28.8 1.0 Val 12 12.1 0.2 Met 7 6.0 0.6 Ile 8 6.7 0.1 Leu 31 26.9 0.7 Tyr 7 7.8 0.2 Phe 5 5.7 0.1 His 13 12.8 0.1 Lys 10 10.8 0.2 Arg 20 20.3 0.2 Trp 4 nd nd Ethanolamine -- 2.6 0.4

Assumed u-PAR sequence after processing (Leu.sub.1 -Ala.sub.284) B) Predicted Determined after Amino acid from cDNA acid hydrolysis SD

Asp + Asn 29 29.8 0.4 Thr.sup.b 20 20.0 0.5 Ser.sup.b 24 23.6 0.4 Glu + Gln.sup.c 36 38.1 1.2 Pro 9 10.2 0.3 Gly 26 26.8 1.0 Ala 8 7.6 0.1 Cys.sup.d 28 26.3 0.9 Val 12 11.0 0.2 Met 6 5.5 0.5 Ile 7 6.1 0.1 Leu 24 24.5 0.6 Tyr 7 7.1 0.1 Phe 5 5.2 0.1 His 12 11.6 0.1 Lys 10 9.9 0.2 Arg 19 18.6 0.2 Trp 2 nd nd Ethanolamine -- 2.4 0.4

Footnotes to Table 5 .sup.a. Purified uPAR was prepared for amino acid analysis as described i the legend to FIG. 12 A-B. The presented values represent the average of independent determinations. The data were normalized relative to all amin acids, except tryptophan, assuming a total number of 309 residues for the nascent uPAR and 282 for the fully processed protein (omitting 4 and 2 tryptophan residues, respectively). Amino acid numbering was based upon the cDNA sequence for uPAR without the signal sequence (Example 3). .sup.b. The values for these hydroxyamino acids were corrected for decomposition during hydrolysis Ser (5%) and Thr (10%). .sup.c. A slight overestimation is expected due to the formation of pyroglutamic acid in the amino acid standard mixture. .sup.d. In one sample cysteine was derivatized before hydrolysis by in situ alkylation using iodoacetamide and subsequently quantified as Scarboxymethylcysteine after acid hydrolysis. In general, the yield of this alkylation procedure is 95% (Ploug, 1989). Otherwise, cysteine was derivatized during hydrolysis in the presence of 3,3dithiodipropionic aci (DTDPA) and quantified as the mixed disulfide compound (Cysx) formed between cysteine and DTDPA. .sup.e. nd = not determined. .sup.f. SD = standard deviation (absolute number of residues).

Detailed Description Paragraph Table (12):

SEQUENCE LISTING (1) GENERAL INFORMATION: (iii) NUMBER OF SEQUENCES: 21 (2) INFORMATION FOR SEQ ID NO:1: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 16 amino acids (B) TYPE:

amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
LeuXaaXaaMetGlnXaaLysThrAsnGlyAspXaaArgValGluGlu 151015 (2) INFORMATION FOR SEQ ID NO:2: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2: LeuXaaCysMetGlnCysLysThrAsnGlyAspCysArgValGluGlu 151015 HisAlaLeuGlyGlnXaaLeuXaaArgThrThrIleValXaa 202530 (2) INFORMATION FOR SEQ ID NO:3: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 92 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3: LeuArgCysMetGlnCysLysThrAsnGlyAspCysArgValGluGlu 151015 CysAlaLeuGlyGlnAspLeuCysArgThrThrIleValArgLeuTrp 202530
GluGluGlyGluGluLeuGluLeuValGluLysSerCysThrHisSer 354045
GluLysThrAsnArgThrLeuSerTyrArgThrGlyLeuLysIleThr 505560
SerLeuThrGluValValCysGlyLeuAspLeuCysAsnGlnGlyAsn 65707580
SerGlyArgAlaValThrTyrSerArgSerArgTyr 8590 (2) INFORMATION FOR SEQ ID NO:4: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 99 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4: LeuGluCysIleSerCysGlySerSerAspMetSerCysGluArgGly 151015
ArgHisGlnSerLeuGlnCysArgSerProGluGluGlnCysLeuAsp 202530
ValValThrHisTrpIleGlnGluGlyGluGluGlyArgProLysAsp 354045
AspArgHisLeuArgGlyCysGlyTyrLeuProGlyCysProGlySer 505560
AsnGlyPheHisAsnAsnAspThrPheHisPheLeuLysCysCysAsn 65707580
ThrThrLysCysAsnGluGlyProIleLeuGluLeuGluAsnLeuPro 859095 GlnAsnGly (2) INFORMATION FOR SEQ ID NO:5: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 90 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
ArgGlnCysTyrSerCysLysGlyAsnSerThrHisGlyCysSerSer 151015
GluGluThrPheLeuIleAspCysArgGlyProMetAsnGlnCysLeu 202530
ValAlaThrGlyThrHisGluProLysAsnGlnSerTyrMetValArg 354045
GlyCysAlaThrAlaSerMetCysGlnHisAlaHisLeuGlyAspAla 505560
PheSerMetAsnHisIleAspValSerCysCysThrLysSerGlyCys 65707580
AsnHisProAspLeuAspValGlnTyrArg 8590 (2) INFORMATION FOR SEQ ID NO:6: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 36 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6: GCCAGACTGTGGGGAGGCACTCTCCTCTGGACCTAA36 (2) INFORMATION FOR SEQ ID NO:7: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 11 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7: AlaArgLeuTrpGlyGlyThrLeuLeuTrpThr 1510 (2) INFORMATION FOR SEQ ID NO:8: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 11 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (genomic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8: CCANNNNNTGG11 (2) INFORMATION FOR SEQ ID NO:9: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9: AGAGT5 (2) INFORMATION FOR SEQ ID NO:10: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10: ACAGT5 (2) INFORMATION FOR SEQ ID NO:11: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11: AGACT5 (2) INFORMATION FOR SEQ ID NO:12: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12: ACTGT5 (2) INFORMATION FOR SEQ ID NO:13: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 14 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13: CTAGTCTAGACTAG14 (2) INFORMATION FOR SEQ ID NO:14: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 24 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
AGACTCTAGTCTAGACTAGACTGT24 (2) INFORMATION FOR SEQ ID NO:15: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 17 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15: GACCTGGATATCCAGTA17 (2) INFORMATION FOR SEQ ID NO:16: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 25 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16: GluProGlyAlaAlaThrLeuLysSerValAlaLeuProPheAlaIle 151015

AlaAlaAlaAlaLeuValAlaAlaPhe 2025 (2) INFORMATION FOR SEQ ID NO:17: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 26 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17: CysLysAspSerSerIleValLeuThrLysLysPheAlaLeuThrVal 151015 ValSerAlaAlaPheValAlaLeuLeuPhe 2025 (2) INFORMATION FOR SEQ ID NO:18: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 32 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18: ThrThrAspAlaAlaHisProGlyArgSerValValProAlaLeuLeu 151015 ProLeuLeuAlaGlyThrLeuLeuLeuLeuGluThrAlaThrAlaPro 202530 (2) INFORMATION FOR SEQ ID NO:19: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 29 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19: ValSerAlaSerGlyThrSerProGlyLeuSerAlaGlyAlaThrVal 151015 GlyIleMetIleGlyValLeuValGlyValAlaLeuIle 2025 (2) INFORMATION FOR SEQ ID NO:20: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 34 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20: ValLysCysGlyGlyIleSerLeuLeuValGlnAsnThrSerTrpLeu 151015 LeuLeuLeuLeuLeuSerLeuSerPheLeuGlnAlaThrAspLysIle 202530 SerLeu (2) INFORMATION FOR SEQ ID NO:21: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 34 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21: TyrArgSerGlyAlaAlaProGlnProGlyProAlaHisLeuSerLeu 151015 ThrIleThrLeuLeuMetThrAlaArgLeuTrpGlyGlyThrLeuLeu 202530

Other Reference Publication (23):
Nelles, L., et al., "Characterization of Recombinant Human Single Chain Urokinase-type Plasminogen Activator Mutants Produced by Site-specific Mutagenesis of Lysine 158," J. Biol. Chem., 262(12):5682-89, (1987).

CLAIMS:

1. An expression vector which is capable of replicating in a host cell, said vector comprising

(a) a coding sequence which encodes a polypeptide, soluble in aqueous solution, and having urokinase plasminogen activator (UPA) binding activity, said polypeptide comprising a UPA-binding domain having an amino acid sequence which (i) is identical to SEQ ID NO:3, (ii) is identical to the amino acid sequence of a UPA-binding 16 kDa glycosylated chymotryptic fragment of the mature uPA receptor protein having the amino acid sequence shown in sequence (A), or (iii) differs in amino acid sequence from (i) or (ii) above only by a single conservative amino acid substitution;

(b) a stop codon immediately following said coding sequence; and

(c) a promoter functional in said host cell and operably linked to said coding sequence;

with the proviso that said vector does not comprise any sequence encoding a mature UPA receptor having the amino acid sequence shown in Sequence A or its natural precursor.

2. The vector of claim 1 wherein the polypeptide comprises a UPA-binding domain which is identical to SEQ ID NO:3, or which differs therefrom solely by a single conservative amino acid substitution of amino acids.

4. The vector of claim 1 wherein the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A, or differs therefrom only by a carboxy terminal truncation and/or by one or more conservative substitutions of amino acids.

5. The expression vector of claim 4 in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A, or differs therefrom solely by one or more conservative substitutions.

6. The expression vector of claim 4 in which the amino acid sequence of the

polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A, or to a fragment thereof which retains at least residues 1-92.

7. The expression vector of claim 4 in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A.

8. The expression vector of claim 4 in which the amino acid sequence of the polypeptide is identical to SEQ ID NO:3.

9. The vector of claim 1 where said polypeptide comprises an amino acid sequence corresponding to the amino acid sequence of a 16 kDa chymotryptic fragment of the UPA receptor which has UPA binding activity, or to a sequence differing from that of said fragment by a single conservative substitution.

13. The method of claim 12 wherein the polypeptide comprises a UPA-binding domain which is identical to SEQ ID NO:3, or which differs therefrom solely by a single conservative amino acid substitution of amino acids.

15. The method of claim 12, wherein the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A, or differs therefrom only by (A) a carboxy terminal truncation (B) one or more conservative substitutions of amino acids, or (C) both (A) and (B) above.

16. The method of claim 15, in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A, or differs therefrom only by one or more conservative substitutions.

17. The method of claim 15, in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A, or to a fragment thereof which retains at least residues 1-92.

18. The method of claim 15, in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A.

19. The method of claim 15, in which the amino acid sequence of the polypeptide is identical to SEQ ID NO:3.

20. The method of claim 12 where said polypeptide comprises an amino acid sequence corresponding to the amino acid sequence of a 16 kDa chymotryptic fragment of the UPA receptor which has UPA binding activity, or to a sequence differing from that of said fragment by a single conservative substitution.

L7: Entry 8 of 12 File: USPT Mar 30, 1999

DOCUMENT-IDENTIFIER: US 5888964 A
TITLE: Method for increasing placental blood flow

Brief Summary Text (4):
AT-III is one kind of glycoproteins belonging to .alpha..sub.2 -globurin present in blood plasma and has a molecular weight of 65,000-68,000. It has an inhibitory activity on blood coagulating protease and strongly inhibits coagulative activity of thrombin.

Detailed Description Text (10):
Examples of the additives and stabilizers include saccharides such as monosaccharides (e.g., glucose and fructose), disaccharides (e.g., sucrose, lactose and maltose) and sugar alcohols (e.g., mannitol and sorbitol); organic acids such as citric acid, malic acid and tartaric acid and salts thereof (e.g., sodium salt, potassium salt and calcium salt); amino acids such as glycine, aspartic acid and glutamic acid and salts thereof (e.g., sodium salt); surfactants such as polyethylene glycol, polyoxyethylene-polyoxypropylene copolymer and polyoxyethylenesorbitan fatty acid ester; heparin; and albumin.

Detailed Description Text (20):
Immediately after the completion of the administration of AT-III or physiological saline, physiological saline containing .sup.57 Co-labeled radioactive microspheres (about 100,000, diameter 15.5.+-.0.1 .mu.m) was injected from the ventriculus sinister. This microsphere solution was a solution of the above-mentioned microspheres homogeneously dispersed in physiological saline (0.5 ml) containing 0.01% Tween 80 by ultrasonic treatment. The arterial blood to be the control was taken from the right femoral artery at 0.458 ml/min over one minute starting from 5 seconds before the injection of the microsphere solution. Immediately after taking the control blood sample, the rats were subjected to thoracotomy and perfusation with physiological saline (250 ml). The residual blood in the blood vessels was removed to the greatest possible extent. The placenta was removed, wiped on the surface and weighed.

Detailed Description Text (26):
A plasmin inhibitor tranexamic acid (200 mg/kg) was intravenously administered to suppress fibrinolysis in the animals that underwent the same surgery as in Experimental Example 1. Then, .sup.125 I-fibrinogen (0.5 .mu.Ci) dissolved in physiological saline was intravenously administered.

L7: Entry 11 of 12 File: USPT Jul 12, 1994

DOCUMENT-IDENTIFIER: US 5328996 A
TITLE: Bacterial plasmin receptors as fibrinolytic agents

Brief Summary Text (7):
The biochemical interactions occurring at cell surfaces between bacterial membranes and their surroundings are complex and not well understood. Certain bacterial surface structures and secreted products have been suggested to contribute to tissue invasion. One of these secreted products, streptokinase, is a plasminogen activator and converts the host zymogen plasminogen to the active protease, plasmin (Siefring, G. E., F. J. Castellino [1976] J. Biol. Chem. 251:3913-3920). Although classically described as the enzyme responsible for fibrin degradation, plasmin is a serine protease with trypsin-like specificity and has activity for a broad range of substrates. Plasmin can degrade several mammalian extracellular matrix proteins, such as fibronectin and laminin, and can enhance collagenase activity (Liotta, L. A., R. H. Goldfarb, R. Brundage [1981] Cancer Res. 41:4629-4636). Therefore, the ability to generate and capture active plasmin may contribute to the invasive propensity of certain streptococcal strains. The interaction of plasmin with group A streptococci has high affinity (K.sub.d, 10.sup.-10 M) and is specific for plasmin, with no significant binding demonstrated for structurally related proteins (Broeseker, T. A., M. D. P. Boyle, R. Lottenberg [1988] Microb. Pathog. 5:19-27; DesJardin, L. E., M. D. P. Boyle, R. Lottenberg [1989] Thromb. Res. 55:187-193).

Brief Summary Text (8):
As described herein, the plasmin receptor of the subject invention has significant similarity to the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH). GAPDH is a key enzyme involved in glucose metabolism and has been the subject of many genetic studies. Multiple copies of GAPDH genes have been identified for mammals, with many described as pseudogenes (Piechaczyk, M., J. M. Blanchard, S. Riaad-El Sabouty, C. Dani, L. Marty, P. Jeanteur [1984] Nature 312:469-471). Multiple GAPDH genes have also been identified for E. coli, Trypanosoma brucei, Saccharomyces cerevisiae, and Drosophila melanogaster (Alefounder, P. R., R. N. Perham [1989] Mol. Microbiol. 3:723-732; Holland, J. P., L. Banieniec, C. Swimmer, M. J. Holland [1983] J. Biol. Chem. 258:5291-5299; Michels, P. A. M., M. Marchand, L. Kohl, S. Allert, R. K. Wierenga, F. R. Opperdoes [1991] Eur. J. Biochem. 198:421-428; Tso, J. Y., X. -H. Sun, R. Wu [1985] Nucleic Acids Res. 17:1251). E. coli and T. brucei each have two GAPDH genes with significant differences in deduced amino acid sequence (Alefounder et al., supra; Michels et al., supra); however, the translated product of the second E. coli GAPDH gene has not been reported. One of the trypanosomal isoenzymes is localized in the glycosome, a specialized metabolic organelle, while the other GAPDH is found in the cytoplasm (Lambier, A. -M., A. M. Loiseau, D. A. Kuntz, F. M. Vellieux, P. A. M. Michels, F. R. Opperdoes [1991] Eur. J. Biochem. 198:429-435).

Brief Summary Text (9):
In addition to its usual intracellular location, GAPDH has been identified on the surface of hematopoietic cells and Schistosoma mansoni, an invasive parasite (Allen, R. W., K. A. Trach, J. A. Hoch [1987] J. Biol. Chem. 262:649-653; Goudot-Crozel, V., D. Caillol, M. Djabali, A. J. Dessein [1989] J. Exp. Med. 170:2065-2080). Allen and Hoover ([1985] Blood 65:1045-1055) characterized a membrane-associated 37,000-M.sub.r protein expressed by the erythroleukemic cell line K562. Peptide mapping and molecular cloning studies revealed the protein to be homologous to GAPDH (Allen, Trach, and Hoch, supra). A similar finding has been reported for the blood

fluke responsible for abdominal schistosomiasis (Goudet-Crozel et al., supra). A 37,000-M.sub.r surface immunogen of S. mansoni was characterized by isolating the cDNA encoding the protein. The deduced amino acid sequence had significant homology to that of human GAPDH. Like the recombinant plasmin receptor protein (Plr), neither of these surface proteins had domains corresponding to previously described membrane-anchoring structures (Blobel, G. [1980] Proc. Natl. Acad. Sci. USA 77:1496-1500; Ferguson, M. A. J., A. F. Williams [1988] Annu. Rev. Biochem. 57:285-320). Interestingly, Hekman et al. (Hekman, W. E., D. T. Dennis, J. A. Miernyk [1990] Mol. Microbiol. 4:1363-1369), while studying the expression of recombinant plant GAPDH in E. coli, were able to target the protein to the outer membrane by genetically fusing the signal sequence of E. coli OmpA to Ricinus communis GAPDH. Pancholi et al. have recently reported the isolation of a 39 kD surface protein with GAPDH activity from a group A streptococci (Pancholi, V., V. A. Fischetti [1992] "A Novel Multifunctional Surface Protein (MFG) of group A Streptococci," Abstract No. B-252, Abstracts of the General Meeting 1992:68).

Detailed Description Text (2):
SEQ ID NO. 1 shows the composite DNA and translated amino acid sequences of the plasmin receptor protein of the subject invention.

Detailed Description Text (3):
SEQ ID NO. 2 is the translated amino acid sequence of the plasmin receptor protein of the subject invention.

Detailed Description Text (12):
We have isolated and analyzed plr, the gene encoding a group A streptococcal plasmin receptor. By screening a .lambda. gt11 expression library with antiplasmin receptor antibodies, we identified a plasmin receptor gene within a 2.7-kb streptococcal DNA fragment. This fragment was subcloned into a low-copy-number plasmid, and the receptor protein was stably expressed in E. coli under the control of putative streptococcal promoter elements. The recombinant receptor protein demonstrated immunoreactivity and plasmin-binding activity. We determined the nucleotide sequence for plr and upstream elements of the structural gene. An open reading frame of 1,008 bp was identified. The 40.5% G+C content of plr was slightly higher than the 35 to 39% reported for group A streptococcal chromosomal DNA (Hardie, J. M. [1986] "Genus Streptococcus," pp. 1043-1071, In J. G. Holt et al. (eds.), Bergey's manual of systematic bacteriology, Williams & Wilkins, Baltimore). The deduced amino acid sequence was identical for 74 amino acid residues at the N terminus as well as three cyanogen bromide fragments obtained from the native streptococcal protein. The amino acid sequence obtained for the streptococcal receptor protein revealed that the initial methionine residue is cleaved.

Detailed Description Text (13):
The deduced amino acid sequence for the recombinant plasmin receptor protein (Plr) was compared with published sequences for other proteins. Plr exhibits significant similarity to the glycolytic enzyme GAPDH, reported for a number of prokaryotic and eukaryotic organisms. The best match was with B. subtilis (56% identical and 73% conserved amino acid residues). GAPDH from streptococci has not been isolated or characterized, and the relationship of the plasmin receptor to the glycolytically active enzyme remains to be defined. However, the extensive amino acid homology and similar hydropathy plots for Plr and B. subtilis GAPDH strongly suggest that Plr is a member of the GAPDH family of proteins. Furthermore, preliminary analysis of the recombinant protein revealed that Plr has GAPDH enzymatic activity.

Detailed Description Text (17):
In addition to the plasmin receptor amino acid sequence disclosed herein, the subject invention further comprises equivalent plasmin receptor proteins (and nucleotide sequences coding for equivalent proteins) having the same or similar biological activity of the plasmin receptor exemplified herein. These equivalent proteins may have amino acid homology with the protein disclosed and claimed herein. This amino acid homology will typically be greater than 75%, preferably be greater than 90%, and most preferably be greater than 95%. The amino acid homology will be highest in certain critical regions of the protein which account for biological activity or are involved in the determination of three-dimensional configuration which ultimately is responsible for the biological activity. In this regard, certain

amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: nonpolar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Table 1 provides a listing of examples of amino acids belonging to each class.

Detailed Description Text (18):
In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not significantly detract from the biological activity of the protein. It has been shown that proteins of identified structure and function may be constructed by changing the amino acid sequence if such changes do not alter the protein secondary structure (Kaiser, E. T. and Kezdy, F. J. [1984] Science 223:249-255). Thus, the subject invention includes mutants of the amino acid sequence depicted herein which do not alter the protein secondary structure, or if the structure is altered, the biological activity is substantially retained.

Detailed Description Text (20):
It should be apparent to a person skilled in this art that genes coding for receptor-binding proteins can be identified and obtained through several means. The specific genes may be obtained from a culture depository as described herein. Alternatively, these genes, or portions thereof, may be constructed synthetically, for example, by use of a gene machine. Variations of these genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as Bal31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which code for active fragments may be obtained using a variety of other restriction enzymes. Proteases may be used to directly obtain active fragments of these proteins.

Detailed Description Text (24):
Radioiodination of proteins. Human plasminogen isolated from plasma by chromatography on lysine-Sepharose (Lottenberg, R., F. R. Dolly, C. S. Kitchens [1985] Am. J. Hematol. 19:181-193) and streptococcal protein G (Calbiochem, San Diego, Calif.) were labeled with .sup.125 I (Amersham Corp., Arlington Heights, Ill.) by using a mild lactoperoxidase reaction with Enzymo-beads (Bio-Rad, Richmond, Calif.) (McCoy II, E., C. C. Broder, R. Lottenberg [1991] J. Infect. Dis. 164:515-521). Plasmin was generated from radiolabeled plasminogen as previously described (Broder, C. C., R. Lottenberg, G. O. von Mering, K. H. Johnston, M. D. P. Boyle [1991] J. Biol. Chem. 266:4922-4928).

Detailed Description Text (27):
Screening of the streptococcal library. The resulting non-amplified .lambda. gt11 library was diluted in 10 mM Tris-2.5 mM MgSO.sub.4 -0.01% gelatin-0.1M NaCl, pH 7.5, and used to infect E. coli Y1090, yielding a density of 200 to 400 plaques per plate. The infected cells were mixed with 0.45% soft agar, plated on 1.2% L agar supplemented with ampicillin (50 .mu.g/ml), incubated at 42.degree. C. for 3 to 4 hours to induce lysis, and overlaid with nitrocellulose filters impregnated with 10 mM isopropylthiogalactoside (IPTG) to induce the lac promoter. After incubation at 37.degree. C. for approximately 16 hours, the filters were removed, washed, and blocked in 100 mM Tris-300 mM NaCl-5 mM EDTA-0.05% Triton X-100-0.25% gelatin, pH 7.4 (NET-gel). The filters were then incubated with murine antiplasmin receptor antibody (Broder et al. [1991], supra) for approximately 18 hours at room temperature, washed, and then incubated with goat anti-mouse immunoglobulin G (Cappel, Organon Teknika) for 3 to 4 hours. Antigen-antibody complexes on washed filters were detected with .sup.125 I-streptococcal protein G. Autoradiographs were generated by exposing the washed nitrocellulose filters to Kodak XAR-5 film with intensifying screens at -70.degree. C. and then using automated film developing. Immunoreactive plaques were isolated and purified through two additional screenings.

Detailed Description Text (32):
Amino acid sequencing. Mutanolysin-extracted proteins (Broder et al. [1991], supra) from strain 64/14 were subjected to SDS-PAGE. The proteins were electrotransferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore Corp., Bedford, Mass.) by using 10 mM 2-(N-morpholino)ethanesulfonic acid (pH 6.0)-20% methanol as the transfer buffer. Protein bands were stained with Coomassie brilliant blue. The .apprxeq.41,000-M.sub.r protein band, which had previously been shown to bind plasmin, was excised. Microsequencing by automated Edman chemistry was performed with an Applied Biosystems model 470A gas-phase sequencer with an on-line 12A PTH analyzer (Washington University Protein Chemistry Laboratory). Cyanogen bromide fragmentation of the .apprxeq.41,000-M.sub.r protein was performed by immersing the polyvinylidene difluoride membrane-bound protein in 70% formic acid and treating with cyanogen bromide overnight at room temperature. The fragments were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane as described above. Four peptides were identified and sequenced at the University of Florida Interdisciplinary Center for Biotechnology Research Protein Chemistry Core Facility by using an Applied Biosystems model 470 sequencer with an on-line PTH analyzer.

Detailed Description Text (36):
Plasminogen was prepared from human plasma by chromatography on lysine sepharose (Sigma Chem. Co., St. Louis, Mo. U.S.A.) and molecular sieving chromatography on Sephadex G-100 (Lottenberg, R., F. R. Dolly, C. S. Kitchens [1985] Am. J. Hematol. 19:181-193). The purified protein appeared as a single band on a silver stain of an SDS-polyacrylamide gel electrophoresis. A given concentration of isolated plasminogen following activation with streptokinase demonstrated the predicted theoretical amidolytic activity, thereby confirming the purity of the isolated human plasminogen.

Detailed Description Text (42):
Plasmin was generated from radiolabeled plasminogen by incubation with urokinase (20 units/ml, Sigma Chemical Co., St. Louis, Mo., U.S.A.) in VBS-gel that contained 0.02M lysine. Conversion was maximal after 30 min at 37.degree. C. The conversion of the single chain zymogen molecule to heavy and light chains was monitored, following reduction, on SDS-PAGE using the method of Laemmli as described previously (Lottenberg, R., C. C. Broder, M. D. P. Boyle [1987] Infect. Immmun. 55: 1914-1918). Greater than 95% of plasminogen was consistently converted to plasmin. The specific activity of labeled plasmin was therefore essentially the same as labeled plasminogen.

Detailed Description Text (45):
The group A.beta.-hemolytic streptococcal strain 64 had been previously subjected to mouse passage (Reis, K. J., M. Yarnall, E. M. Ayoub, M. D. P. Boyle [1984] Scand. J. Immunol. 20: 433-439). The parent strain (64/P), as well as strains isolated after three (64/3) and fourteen (64/14) passages, were grown in Todd-Hewitt broth (DIFCO, Detroit, Mich.) overnight in phosphate-buffered saline (PBS), pH 7.4, containing 0.05% Tween-20 and 0.02% sodium azide. The bacteria were heat killed at 80.degree. C. for 5 min, a treatment that did not alter their plasmin binding potential, but eliminated the production of soluble plasminogen activators which would interfere with these studies. The suspension was centrifuged, the pellet washed twice with PBS 0.02% sodium azide. Samples were stored at -20.degree. C. The concentration of a bacterial suspension was determined by counting bacterial chains in a Neubauer hemacytometer (Fisher Scientific, Orlando, Fla., U.S.A.)

Detailed Description Text (48):
To assess the effect of pH on the bacterium: plasmin (ogen) interaction, 50 .mu.m of labeled plasminogen of plasmin (approximately 2.times.10.sup.4 cpm) were added to 1.0 ml of VBS containing 0.05% Tween-20 adjusted to the appropriate pH. After 15 min at room temperature, 50 .mu.m of VBS containing approximately 10.sup.7 bacteria (strain 64/14) were added and the mixture was incubated at 37.degree. C. for 15 min. The bacterial suspensions were centrifuged at 1000 g for 7 min to separate bacteria from unbound labeled proteins and the pellets were washed twice with 2 ml of VBS at the appropriate pH. The radioactivity associated with the bacterial pellet in duplicate experiments was measured using a Beckman 5500 autogamma counter.

Detailed Description Text (50):
To assess the effect of ionic strength on the bacterium:plasmin (ogen) interaction, similar studies were carried out in solutions containing different concentrations of NaCl with 0.05% Tween-20. The bacterial pellets were washed in the appropriate NaCl concentration to remove unbound labeled proteins.

Detailed Description Text (57):
Inhibition of Binding by Amino Acids

Detailed Description Text (58):
Labeled plasmin (100 .mu.l containing approximately 2.times.10.sup.4 cpm) was added to 200 .mu.l VBS-gel containing varying concentrations of epsilon-aminocaproic acid (EACA), lysine, or arginine, and incubated at 37.degree. C. for 15 min. The pH of each solution was 7.0. One hundred .mu.l of VBS-gel containing 10.sup.7 bacteria (strain 64/14) were then added and the mixture was incubated at 37.degree. C. for 15 min. The bacterial suspensions were centrifuged at 1000 g for 7 min and washed twice with 2 ml of VBS-gel containing the same concentration of amino acid present during the incubation period. The percent inhibition of binding was calculated for duplicate experiments by comparison with binding in VBS-gel alone.

Detailed Description Text (59):
The ability of EACA, lysine, or arginine to dissociate bound plasmin from the bacteria was examined in the following manner. Labeled plasmin was incubated with 10.sup.7 bacteria in VBS-gel containing no amino acid at 37.degree. C. for 15 min. The bacteria were pelleted by centrifugation and washed twice with 2 ml on VBS-gel. After determining the radioactivity associated with the bacteria, the pellets were resuspended in solutions of VBS-gel containing varying concentrations of amino acid (pH 7.0) as described above. The mixtures were incubated at 37.degree. C. for 15 min and washed twice with VBS-gel containing the appropriate amino acid concentration. The radioactivity associated with the bacteria in duplicate experiments was again measured and the percentage dissociated was calculated.

Detailed Description Text (60):
Binding of plasmin to the group A streptococcus 64/14 was inhibited by each amino acid in a concentration dependent fashion. Fifty percent inhibition of binding of plasmin to the bacteria was observed at an EACA concentration of 0.15 mM a lysine concentration of 2.0 mM, and an arginine concentration of 25 mM. In similar studies, plasmin was prebound to the group A streptococcus and a concentration dependent elution of bound radiolabel was observed on incubation with EACA, lysine, or arginine. The concentration of amino acid required to elute 50% of the bound plasmin was approximately equivalent to that required to inhibit plasmin binding by 50%.

Detailed Description Text (65):
Plasmin which had been bound to and eluted from strain 64/14 by treatment with lysine was also examined in similar binding studies. Eluted plasmin was obtained by incubating 2 ml of stock 10% wet weight/volume bacterial suspension (strain 64/14) with approximately 20 .mu.g of labeled plasmin at room temperature for 45 min. This suspension was centrifuged at 1000 g for 10 min and washed once with 10 ml of VBS-gel, and the radioactivity associated with the bacterial pellet was measured. The pellet was then resuspended in VBS-gel containing 20 mM lysine and incubated at room temperature for 30 min. The suspension was centrifuged and the supernatant recovered. Approximately 90% of the radioactivity originally associated with the bacterial pellet was dissociated by the lysine treatment. The dissociated plasmin in the supernatant was the subjected to gel filtration of a G-25 column to separate lysine from plasmin. Fractions containing plasmin were collected and stored at -20.degree. C.

Detailed Description Text (69):
Bacterial extracts, chromatography fractions or standards were loaded into the wells of a dot-blotting manifold in 50-200 .mu.l aliquots. Commercially available group C streptokinase was used as a positive plasmin binding control in each assay. All wells were washed twice with 200 .mu.l aliquots of PBS-azide and vacuum drained. All samples were assayed in duplicate. Dot blots were blocked in 5.0 mM sodium diethylbarbiturate, 0.14M NaCl, 0.5% gelatin, 0.15% Tween 20, 0.004% NaN.sub.3 pH 7.3. The blots were probed for 3-4 hours at room temperature in the blocking buffer

containing 2.0 mM PMSF and .sup.125 I-labeled human plasmin at 3.times.10.sup.4 cpm/ml. The probed blots were then washed in 0.01M EDTA pH 7.3, containing 0.5M NaCl 0.25% gelatin, 0.15% Tween 20, and 0.004% NaN.sub.3. Autoradiographs were generated by exposing the nitrocellulose blots to Kodak XAR-5 film with an intensifying screen for 15-24 hours at -70.degree. C. followed by automated film developing.

Detailed Description Text (72):
Gels intended for Western blotting were equilibrated in 25 mM Tris, 0.2M glycine pH 8.0 containing 20% v/v methanol (electroblot buffer) for 25 minutes. Protein blotting, from SDS-PAGE gels, was performed using the `Trans-Blot SD Semi-Dry` electrophoretic transfer cell (Bio Rad, Richmond, Calif.). Blots were blocked as described for the dot-blot procedure, and probed for 3-4 hours at room temperature with radiolabeled human plasmin in either the presence or absence of 1.0 mM EACA, to identify functionally active protein bands. In studies of antigenic properties of these proteins, blots were probed with rabbit anti-plasmin receptor antibody or anti-group C streptokinase antibody by incubation with 4.3 .mu.g IgG per ml of probing solution (approximately a 1:3000 dilution of antisera) for three hours and probed with .sup.125 I-streptococcal protein G containing 3.times.10.sup.4 cpm/ml. For probing with mouse monoclonal antibodies specific for epitopes on group C streptokinase, blots were probed with a 1:100 dilution of the monoclonal antibody stock solution for three hours, followed by probing with goat antibody specific for mouse IgG at 1.0 .mu.g/ml, followed by probing with .sup.125 I-streptococcal protein G containing 3.times.10.sup.4 cpm/ml. Blots were then washed with 0.01M EDTA pH 7.3, containing 1.0M NaCl, 0.25% gelatin, 0.15% Tween 20. Autoradiographs were generated by exposing the nitrocellulose blots to Kodak XAR-5 film with an intensifying screen for 15-24 hours at -70.degree. C. followed by automated film developing.

Detailed Description Text (76):
This procedure is a modification of the method described by Yarnall, M. and M. D. P. Boyle (1986) "Isolation and partial characterization of a type II Fc receptor from a group A streptococcus," Mol. Cell. Biochem. 70:57-66. Approximately 1.0 g wet weight of bacteria was suspended in 5.0 ml of 20 mM KH.sub.2 PO.sub.4, 1.0 mM EDTA, 0.02% NaN.sub.3 pH 7.0 containing 2.0 mM PMSF, 10 .mu.g/ml DNAse I and 50 .mu.g/ml mutanolysin. The suspension was vortexed and placed at 37.degree. C. for 4 hours with periodic mixing. Supernatants were collected following centrifugation to remove bacteria and debris. For these studies a commercial preparation of mutanolysin was further purified according to the method described by Siegal et al. (Siegal, J. L., S. F. Hurst, E. S. Liberman, S. E. Coleman, A. S. Bleiweis [1981] "Mutanolysin-induced spheroplasts of Streptococcus mutans are true protoplasts," Infect. Immun. 31:303-815) to remove contaminating protease.

Detailed Description Text (79):
Human plasminogen at a concentration of approximately 5.6.times.10.sup.-5 M was activated to plasmin by incubating the sample in the presence of an approximately 60 fold lower molar concentration of urokinase. The reaction was carried out with constant agitation for one hour at 37.degree. C. in a reaction volume of 10 ml of 0.05M Tris, 0.15M NaCl pH 7.4 containing 40 mM lysine. A 50 .mu.l aliquot was removed and analyzed by SDS-PAGE under reduced conditions to determine the extent of conversion of the single chain plasminogen molecule to the two chain plasmin form. The remainder of the reaction mixture was flash frozen, and stored at -70.degree. C. Preparations in which complete conversion of plasminogen to plasmin was observed were then reacted with constant rotation with a 5 fold molar excess of D-valyl-I-phenylalanyl-L-lysine chloromethyl ketone at ambient temperature with constant rotation. The enzymatically inactive plasmin was then concentrated by ammonium sulfate precipitation (4.0 g/10 ml), dialyzed at 4.degree. C. against 0.1M MOPS buffer, pH 7.3, containing 0.02% sodium azide. The dialyzed inactive plasmin was chromatographed on a Superose 6 column (Pharmacia, Piscataway, N.J.) in 0.1M MOPS buffer, pH 7.3.

Detailed Description Text (83):
The Affi-Prep 10-Plasmin matrix was placed in an HR 10/10 column attached to a Pharmacia FPLC chromatography system. The column was equilibrated at room temperature in 0.05M Na.sub.2 HPO.sub.4, 0.15M NaCl, 1.0 mM benzamidine HCl, 0.02% sodium azide pH 7.2 (equilibration buffer). Approximately 1 or 2 ml of supernatant from the mutanolysin extraction of bacterial strain 64/14 was loaded onto the

column. The column was then washed with the equilibration buffer until the OD.sub.280 returned to base line. The column was then eluted with a 50 ml linear gradient of 0.0M-0.1M L-Lysine in equilibration buffer, or eluted in a single step using equilibration buffer containing 0.1M L-Lysine. The absorbance at 280 nm was continuously monitored an 1.0 ml fractions were collected. After each affinity purification procedure the column was washed with 20 ml of 2.0M NaCl, followed by 200 ml of equilibration buffer and stored at 4.degree. C.

Detailed Description Text (98):
The mutanolysin extracted plasmin binding activity was subjected to further purification by affinity chromatography using an enzymatically inactivated plasmin affinity matrix prepared as described above. One ml of the mutanolysin extract of strain 64/14 was applied to the plasmin affinity column matrix in 0.05M Na.sub.2 HPO.sub.4, 0.15M NaCl, 1.0 mM benzamidine HCl, and 0.02% NaN.sub.3, pH 7.2. Bound plasmin receptor activity was eluted using a 50 ml linear gradient of 0.0-0.1M L-Lysine in 0.05M Na.sub.2 HPO.sub.4, 0.15M NaCl, 1.0 mM benzamidine HCl, and 0.02% NaN.sub.3, pH 7.2. The absorbance at 280 nm was monitored continuously and 1.0 ml fractions were collected. Fractions eluted from the affinity column were assayed for functional activity using a dot blotting procedure and .sup.125 I-labeled plasmin as the probe. The functional plasmin binding activity was found to bind to the immobilized plasmin matrix and could be eluted selectively with lysine. The recovered functional activity from the column corresponded to the eluted protein peak as detected by measuring absorbance at 280 nm. Identical results were obtained when a single concentration of 0.1M L-lysine was used to elute the bound plasmin receptor activity from the plasmin affinity column.

Detailed Description Text (113):
N-terminal amino acid sequencing of the .apprxeq.41,000-M.sub.r plasmin receptor protein from strain 64/14 was performed, and an unambiguous sequence was obtained for 51 residues. Amino acid sequences were obtained from four peptides (M.sub.r s of 3,000 to 16,000) generated by cyanogen bromide treatment of the .apprxeq.41,000-M.sub.r protein. The sequence of one of these peptides had identity with the N-terminal sequence and allowed assignment of an additional 23 residues. The deduced amino acid sequence of the open reading frame exhibited complete identity with 74 amino acid residues of the native protein, indicating that valine following the ATG initiation codon represents the N terminus of the receptor protein. The sequences of two additional peptides (13 and 27 residues) were also determined and found to correspond to residues 160 to 173 and 186 to 216, respectively. Thus, 114 of the predicted 335 amino acid residues encoded by plr have been confirmed by amino acid sequencing of the native streptococcal protein.

Detailed Description Text (117):
The deduced amino acid sequence of Plr was compared with deduced amino acid sequences for genes entered in the EMBL (release 26.0) and GenBank (release 67.0) data bases by using the TFASTA program based on the algorithm of Lipman and Pearson (Lipman, D., W. R. Pearson [1985] Science 227: 1435-1441). Glyceraldehyde 3-phosphate dehydrogenases (GAPDHs) of bacterial origins (Branlant, G., C. Branlant [1985] Eur. J. Biochem. 150: 61-66; Schlaepfer, B. S., W. Portmann, C. Branlant, G. Branlant, H. Zuber [1990] Nucleic Acids Res. 18: 6422; Viaene, A., P. Dhaese [1989] Nucleic Acids Res. 17: 1251) exhibited the greatest homology with Plr. The gram-positive Bacillus subtilis GAPDH demonstrated the highest score. The sequences showed 56% identity and 73% similarity.

Detailed Description Text (118):
Hydropathy plots of Plr and B. subtilis GAPDH were determined as described by Kyte and Doolittle (Kyte, J., R. F. Doolittle [1982] J. Mol. Biol. 157: 105-132). Plr and B. subtilis GAPDH showed similar patterns overall except for differences in the C-terminal portion of the molecules. Common cell wall-spanning and membrane-anchoring motifs have been identified for several gram-positive surface proteins (Fischetti, V. A., V. Pancholi, O. Schneewing [1991] "Common characteristics of the surface proteins from gram-positive cocci, pp. 290-294, In G. M. Dunny et al. (eds.), Genetics and molecular biologic of streptococci, lactococci, and enterococci, American Society for Microbiology, Washington, D.C.). However, no similar regions were identified for Plr. No significant amino acid sequence homology between Plr and streptokinase, the other well-characterized plasmin(ogen)-binding

protein, was identified, supporting our previous biochemical and immunological analyses (Broder et al. [1991], supra).

Detailed Description Text (127):
An additional aspect of controlling potential hemorrhage accompanying administration of this agent may be the intravenous administration of epsilon-aminocaproic acid (tradename AMICAR) or tranexamic acid. Typical regimens of these anti-fibrinolytic agents would provide plasma levels of the lysine analogs to dissociate the plasmin from the bacteria (Broeseker, T. A., M. D. P. Boyle, R. Lottenberg [1988] "Characterization of the interaction of human plasmin with its specific receptor on a group A streptococcus," Microbial Pathogenesis 5:19-27) and enable rapid inactivation of plasmin by its physiological inhibitors .alpha.2-antiplasmin and .alpha.2-macroglobulin. This will also provide an approach to prepare the patient for emergency surgical procedures (e.g., coronary artery bypass grafting or administration of alternative anti-thrombotic agents (e.g., heparin).

Detailed Description Paragraph Table (2):
TABLE 1 Class of Amino Acid Examples of Amino Acids Nonpolar Ala, Val, Leu, Ile, Pro, Met, Phe, Trp Uncharged Polar Gly, Ser, Thr, Cys, Tyr, Asn, Gln Acidic Asp, Glu Basic Lys, Arg, His

Detailed Description Paragraph Table (3):
SEQUENCE LISTING (1) GENERAL INFORMATION: (iii) NUMBER OF SEQUENCES: 2 (2) INFORMATION FOR SEQ ID NO:1: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 1125 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: both (D) TOPOLOGY: circular (ii) MOLECULE TYPE: DNA (genomic) (iii) HYPOTHETICAL: NO (v) FRAGMENT TYPE: N-terminal (vi) ORIGINAL SOURCE: (A) ORGANISM: Streptococcus pyogenes (B) STRAIN: M untypable (C) INDIVIDUAL ISOLATE: 64/14 (vii) IMMEDIATE SOURCE: (B) CLONE: pRL015 (ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 115..1122 (ix) FEATURE: (A) NAME/KEY: matpeptide (B) LOCATION: 115..1122 (C) IDENTIFICATION METHOD: experimental (D) OTHER INFORMATION: /codonstart=115 /function="High-affinity binding of plasmin(ogen)" /product="Streptococcal plasmin receptor" /evidence=EXPERIMENTAL /gene="plr" /number=1 /label=PLR (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1: ATAATA

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GTTCTGTTGAAAGGTTGTTGCAGATGACTGTAAGTAATCTTTTCACAATAGGTA60
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GTAGTTAAAGTTGGTATTAACGGTTTCGGTCGTATCGGACGTCTTGCA165
ValValLysValGlyIleAsnGlyPheGlyArgIleGl yArgLeuAla 51015
TTCCGCCGTATTCAAAACATCGAAGGTGTTGAAGTAACTCGTATCAAT213
PheArgArgIleGlnAsnIleGluGlyValGluValThrA rgIleAsn 202530
GACCTTACAGATCCAAATATGCTTGCACACTTGTTGAAATACGATACA261
AspLeuThrAspProAsnMetLeuAlaHisLeuLeuLysTyrAsp Thr 354045
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ThrGlnGlyArgPheAspGlyThrValGluValLysGluGlyGlyPhe 50 556065
GAAGTAAACGGAAACTTCATCAAAGTTTCTGCTGAACGTGATCCAGAA357
GluValAsnGlyAsnPheIleLysValSerAlaGluArgAspProGlu 707580
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GlyPhePheAlaLysLysGluAlaAlaGluLysHisLeuHisAlaAsn 100105110
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ValIleSerGlyAlaSerCysThrThrAsnCysLeuAlaProMetAla 150155160
AAAGCTCTTCACGATGCATTCGGTATTCAAAAAGGTCTTATGACTACA645
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IleHisAlaTyrThrGlyAspGlnMetIleLeuAspGlyProHisArg 180 185190
GGTGGTGACCTTCGTCGTGCACGCGCTGGTGCTGCAAACATCGTTCCT741
GlyGlyAspLeuArgArgAlaArgAlaGlyAlaAlaAsnIleValPro 1952 00205
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ThrGluAspProIleValSerSerAspIleValGlyValSerTyrGly 275280 285
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GCTCAACTTGTACGTACTCTTGAGTACTTCGCAAAAATTGCTAAA1122
AlaGlnLeuValArgThrLeuGluTyrPheAlaLysIleAlaLys 325330 335 TAA1125 (2) INFORMATION FOR SEQ ID NO:2: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 336 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2: MetVal ValLysValGlyIleAsnGlyPheGlyArgIleGlyArgLeu 151015
AlaPheArgArgIleGlnAsnIleGluGlyValGluValThrArgIle 20 2530
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GluAsnIleAspTrpAlaThrAspGlyValGluIleValLeuGluAla 859095
ThrGlyPhePheAlaLysLysGluAlaAlaGluLysHisLeuHisAla 100105110
AsnGlyAlaLysLysValValIleThrAlaProGlyGlyAsnAspVal 115120125 LysThrValValPheAsn
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ThrValIleSerGlyAlaSerCysThrThrAsnCysLeuAlaProMet 145150155 160
AlaLysAlaLeuHisAspAlaPheGlyIleGlnLysGlyLeuMetThr 165170175
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ArgGlyGlyAspLeuArgArgAlaArgAlaGlyAlaAlaAsnIleVal 195200205 ProAsn
SerThrGlyAlaAlaLysAlaIleGlyLeuValIleProGlu 210215220
LeuAsnGlyLysLeuAspGlyAlaAlaGlnArgValProValProThr 225230 235240
GlySerValThrGluLeuValValThrLeuAspLysAsnValSerVal 245250255
AspGluIleAsnSerAlaMetLysAla AlaSerAsnAspSerPheGly 260265270
TyrThrGluAspProIleValSerSerAspIleValGlyValSerTyr 275280 285
GlySerLeuPheAspAlaThrGlnThrLysValMetGluValAspGly 290295300
SerGlnLeuValLysValValSerTrpTyrAspAsnGluMetSerTyr 305 310315320
ThrAlaGlnLeuValArgThrLeuGluTyrPheAlaLysIleAlaLys 325330335

CLAIMS:

1. Isolated DNA, encoding a plasmin receptor having a molecular weight of about 41 kD, said plasmin receptor consisting essentially of the amino acid sequence set forth in SEQ. ID NO. 2.